

# Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/US04/041668

International filing date: 10 December 2004 (10.12.2004)

Document type: Certified copy of priority document

Document details: Country/Office: US

Number: 60/529,404

Filing date: 12 December 2003 (12.12.2003)

Date of receipt at the International Bureau: 31 January 2005 (31.01.2005)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)



World Intellectual Property Organization (WIPO) - Geneva, Switzerland  
Organisation Mondiale de la Propriété Intellectuelle (OMPI) - Genève, Suisse



# THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

*January 19, 2005*

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM  
THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK  
OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT  
APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A  
FILING DATE.

APPLICATION NUMBER: 60/529,404  
FILING DATE: *December 12, 2003*  
RELATED PCT APPLICATION NUMBER: *PCT/US04/41668*



Certified by

Under Secretary of Commerce  
for Intellectual Property  
and Director of the United States  
Patent and Trademark Office

121203

1719 US PTO

SUBSTITUTE PTO/SB/16 (5-03)

## PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR §1.53(c).

INVENTOR(S)				
Given Name (first and middle if any)	Family Name or Surname	Residence (City and either State or Foreign Country)		
Angela Rodrigo	Hornsten Franco	Stow, MA North Andover, MA		
Additional inventors are being named on the 0 separately numbered sheets attached hereto				
TITLE OF THE INVENTION (500 characters max)				
Novel Sodium Channel				
CORRESPONDENCE ADDRESS				
Direct all correspondence to:				
[X] Customer Number: 26161				
OR				
[ ] Firm or Individual Name				
Address				
Address				
City	State	ZIP		
Country	Telephone	Fax		
ENCLOSED APPLICATION PARTS (check all that apply)				
[X] Specification	Number of Pages	90	[ ] CD(s), Number	
[X] Drawing(s)	Number of Sheets	11	[X] Other (specify)	Acknowledgement postcard
[ ] Application Data Sheet. See 37 CFR 1.76.				
METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT				
<input type="checkbox"/> Applicant Claims small entity status. See 37 CFR 1.27. <input checked="" type="checkbox"/> A check or money order is enclosed to cover the filing fees. <input type="checkbox"/> The Director is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: <span style="border: 1px solid black; padding: 2px;">06-1050</span>			FILING FEE AMOUNT (\$)	
<input type="checkbox"/> Payment by credit card. Form PTO-2036 is attached.			<span style="border: 1px solid black; padding: 2px;">\$80</span>	
The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.				
<input checked="" type="checkbox"/> No. <input type="checkbox"/> Yes, the name of the U.S. Government agency and the Government contract number are:				

Respectfully submitted,

Signature 

Date December 12, 2003

Typed Name Jeffrey D. Yosi, Reg. No. 40,024

Telephone No. (617) 542-5070

Docket No. 13594-041P01

20771072.doc

## CERTIFICATE OF MAILING BY EXPRESS MAIL

Express Mail Label No. EL 983 022 757 US

Date of Deposit December 12, 2003

15535 US PTO  
60529402  
121203

PROVISIONAL APPLICATION FOR PATENT

under

37 CFR §1.53(c)

TITLE: NOVEL SODIUM CHANNEL

APPLICANT: ANGELA HORNSTEN AND RODRIGO FRANCO

CERTIFICATE OF MAILING BY EXPRESS MAIL

Express Mail Label No. EL 983 022 757 US

December 12, 2003

Date of Deposit

## NOVEL SODIUM CHANNEL

### BACKGROUND

All cells rely on the regulated movement of inorganic ions across cell membranes to perform essential physiological functions. Electrical excitability, synaptic plasticity, and signal transduction are examples of processes in which changes in ion concentration play a critical role. In general, the ion channels that permit these changes are proteinaceous pores consisting of one or multiple subunits, each containing two or more membrane-spanning domains. Most ion channels have selectivity for specific ions, primarily  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ , or  $\text{Cl}^-$ , by virtue of physical preferences for size and charge. Electrochemical forces, rather than active transport, drive ions across membranes, thus a single channel may allow the passage of millions of ions per second. Channel opening, or "gating" is tightly controlled by changes in voltage or by ligand binding, depending on the subclass of channel. Ion channels are attractive therapeutic targets due to their involvement in many physiological processes, yet the generation of drugs with specificity for particular channels in particular tissue types remains a major challenge.

Voltage-gated ion channels open in response to changes in membrane potential. For example, depolarization of excitable cells such as neurons result in a transient influx of  $\text{Na}^+$  ions, which propagates nerve impulses. This change in  $\text{Na}^+$  concentration is sensed by voltage-gated  $\text{K}^+$  channels which then allow an efflux of  $\text{K}^+$  ions. The efflux of  $\text{K}^+$  ions repolarizes the membrane. Other cell types rely on voltage-gated  $\text{Ca}^{2+}$  channels to generate action potentials. Voltage-gated ion channels also perform important functions in non-excitable cells, such as the regulation of secretory, homeostatic, and mitogenic processes. Ligand-gated ion channels can be opened by extracellular stimuli such as neurotransmitters (e.g., glutamate, serotonin, acetylcholine), or intracellular stimuli (e.g., cAMP,  $\text{Ca}^{2+}$ , and phosphorylation).

Sodium ( $\text{Na}^+$ ) channels include voltage-gated and non-voltage-gated classes. Voltage-gated  $\text{Na}^+$  channels mediate a transient inward flow of  $\text{Na}^+$  ions required for regeneration of action potential in neurons. Voltage-gated  $\text{Na}^+$  channels can be further subdivided into subtypes; at least nine unique voltage-gated  $\text{Na}^+$  channel  $\alpha$  subunits ( $\text{Na}_v1.1-1.9$ ) have been cloned, and at least three associated  $\beta$  subunits have been cloned. The  $\alpha$  subunits of voltage-gated  $\text{Na}^+$  channels are similar to the  $\alpha$  subunits of voltage-gated  $\text{Ca}^{2+}$  channels, and contain four repeat regions, each containing six transmembrane domains. Voltage-gated  $\text{Na}^+$  channels are expressed

in brain, muscle, heart, spinal cord, uterus, and sensory neurons. Alpha subunits associate with auxiliary subunits that regulate the function of the channels for example, by modifying the gating of the  $\alpha$  subunit.

Genetic or pharmacological perturbations in ion channel function can have dramatic clinical consequences. Long QT syndrome, epilepsy, cystic fibrosis, and episodic ataxia are a few examples of heritable diseases resulting from mutations in ion channel subunits. Toxic side affects such as arrhythmia and seizure which are triggered by certain drugs are due to interference with ion channel function (Sirois and Atchison, *Neurotoxicology*, 17(1):63-84, 1996; Keating, M.T., *Science* 272:681-685, 1996). Drugs that modulate ion channel activity have applications in treatment of many pathological conditions, including pain, hypertension, angina pectoris, myocardial ischemia, asthma, bladder overactivity, alopecia, pain, heart failure, dysmenorrhea, type II diabetes, arrhythmia, graft rejection, seizure, convulsions, epilepsy, stroke, gastric hypermotility, psychoses, cancer, muscular dystrophy, and narcolepsy (Coghlan, M.J., *et al.*, *J. Med. Chem.* 44:1627-1653, 2001; Ackerman, M.J., and Clapham, D.E., *N. Eng. J. Med.* 336:1575-1586, 1997). The growing number of identified ion channels and further understanding of their complexity will assist in future efforts at therapies that modify ion channel function.

## SUMMARY

Novel  $\text{Na}^+$  channel subunit polypeptides, nucleic acids, and fragments of the polypeptides and nucleic acids are provided herein. Also provided are methods of using the novel subunit polypeptides, nucleic acids, and fragments thereof.

In one aspect, the invention features an isolated sodium channel type III  $\alpha$  subunit (m $\text{Na}_v$ 1.3  $\alpha$  subunit) polypeptide, wherein the polypeptide includes the amino acid sequence of SEQ ID NO: 2. In one embodiment, the polypeptide essentially consists of the amino acid sequence of SEQ ID NO: 2.

In another aspect, the invention features an isolated m $\text{Na}_v$ 1.3  $\alpha$  subunit polypeptide including at least 10 contiguous amino acids of SEQ ID NO: 2, wherein the polypeptide includes one or more of the following amino acids: isoleucine 289, proline 518, serine 728, serine 1355, asparagine 1909, threonine 1910, and valine 1921.

In another aspect, the invention features an isolated mNa<sub>v</sub>1.3  $\alpha$  subunit nucleic acid molecule that encodes a polypeptide described herein, e.g., a sodium channel type III  $\alpha$  subunit (mNa<sub>v</sub>1.3  $\alpha$  subunit) polypeptide including the amino acid sequence of SEQ ID NO: 2. In one embodiment, the nucleic acid includes the nucleotide sequence of SEQ ID NO: 1. In one embodiment, the nucleic acid molecule essentially consists of the nucleotide sequence of SEQ ID NO: 1. In one embodiment, the nucleic acid is an allele of the nucleic acid sequence of SEQ ID NO: 1.

5 In another aspect, the invention features a fragment of a mNa<sub>v</sub>1.3  $\alpha$  subunit nucleic acid molecule that encodes a polypeptide described herein, e.g., a sodium channel type III  $\alpha$  subunit (mNa<sub>v</sub>1.3  $\alpha$  subunit) polypeptide including the amino acid sequence of SEQ ID NO:2. In one embodiment, the fragment encodes one or more of the following amino acids: isoleucine 289, proline 518, serine 728, serine 1355, asparagine 1909, threonine 1910, and valine 1921.

10 In another aspect, the invention features an expression vector including a nucleic acid encoding a mNa<sub>v</sub>1.3  $\alpha$  subunit described herein, or a fragment thereof, operably linked to a promoter.

15 In another aspect, the invention features a host cell including a nucleic acid encoding a mNa<sub>v</sub>1.3  $\alpha$  subunit described herein, or a fragment thereof.

20 In another aspect, the invention features an agent which preferentially binds to a mNa<sub>v</sub>1.3  $\alpha$  subunit polypeptide, wherein the polypeptide includes the amino acid sequence of SEQ ID NO:2.

25 In another aspect, the invention features an agent which binds selectively to the mNa<sub>v</sub>1.3  $\alpha$  subunit polypeptide, wherein the polypeptide includes the amino acid sequence of SEQ ID NO:2, and wherein the agent does not bind to a sodium channel type I or type II  $\alpha$  subunit polypeptide. In one embodiment, the agent is a small molecule, a nucleic acid, or a protein. In one embodiment, the agent modulates a mNa<sub>v</sub>1.3  $\alpha$  subunit polypeptide activity. In one embodiment, the agent is an antibody or antigen-binding fragment thereof. In one embodiment, the antibody is a polyclonal antibody or a monoclonal antibody.

30 In another aspect, the invention features a pharmaceutical composition including: an agent that binds selectively to a mNa<sub>v</sub>1.3  $\alpha$  subunit polypeptide, wherein the polypeptide comprises the amino acid sequence of SEQ ID NO: 2; and a pharmaceutically acceptable carrier.

In another aspect, the invention features a method for modulating a mNa<sub>v</sub>1.3  $\alpha$  subunit polypeptide activity in a cell, the method including: providing a sodium channel including a mNa<sub>v</sub>1.3  $\alpha$  subunit polypeptide, wherein the mNa<sub>v</sub>1.3  $\alpha$  subunit polypeptide includes the amino acid sequence of SEQ ID NO:2; contacting the channel with an amount of a mNa<sub>v</sub>1.3  $\alpha$  subunit polypeptide modulator effective to modulate an activity of the mNa<sub>v</sub>1.3  $\alpha$  subunit polypeptide. 5 In one embodiment, the modulator is a small molecule, a nucleic acid, or a protein.

In another aspect, the invention features a method for identifying an agent that modulates the activity of a mNa<sub>v</sub>1.3  $\alpha$  subunit polypeptide, the method including: providing a first sodium channel comprising a mNa<sub>v</sub>1.3  $\alpha$  subunit polypeptide, wherein the a mNa<sub>v</sub>1.3  $\alpha$  subunit 10 polypeptide includes the amino acid sequence of SEQ ID NO:2; contacting the channel with a test compound; and evaluating an activity of the sodium channel, wherein a change in activity relative to a reference value is an indication that the compound is an agent that modulates the channel.

15 In one embodiment, the test compound is a small molecule, a peptide, or a nucleic acid. In one embodiment, the sodium channel is contained within a biological sample. In one embodiment, the channel is contacted with multiple test compounds.

In one embodiment, the sample comprises a cell membrane. In one embodiment, the sample comprises a cell. In one embodiment, the cell is a eukaryotic cell. In one embodiment, the cell is *Xenopus* oocyte. In one embodiment, the cell is a mammalian cell.

20 In one embodiment, the activity comprises regulation of sodium concentration. In one embodiment, the evaluating comprises detecting sodium flux. In one embodiment, the contacting occurs under conditions that, in the absence of the test compound, cause a first amount of sodium flux. In one embodiment, the evaluating comprises using a Na<sup>+</sup> flux assay. In one embodiment, the assay uses patch clamp electrophysiology. In one embodiment, the assay 25 uses two electrode voltage clamp electrophysiology. In one embodiment, the assay comprises using a sodium-sensitive dye. In one embodiment, the assay is a high-throughput assay.

In one embodiment, the method further includes the steps of: providing a second sodium channel comprising a mNa<sub>v</sub>1.3  $\alpha$  subunit polypeptide, wherein the mNa<sub>v</sub>1.3  $\alpha$  subunit 30 polypeptide is different that the first mNa<sub>v</sub>1.3  $\alpha$  subunit polypeptide (e.g., the second sodium channel is other than a mNa<sub>v</sub>1.3  $\alpha$  subunit polypeptide comprising the amino acid sequence of

SEQ ID NO: 2); contacting the second sodium channel with the test compound; evaluating the activity of the second sodium channel.

The method can further include comparing the activity of the first sodium channel in the presence of the test compound to the activity of the second sodium channel in the presence of the test compound. In one embodiment, a plurality of sodium channels are provided.

In another aspect, the invention features a method for identifying an agent useful in the treatment of a disorder related to sodium current modulation, the method including: providing a sodium channel comprising a mNa<sub>v</sub>1.3  $\alpha$  subunit polypeptide wherein the polypeptide includes the amino acid sequence of SEQ ID NO:2; contacting the channel with a test compound; and evaluating an activity of the channel, wherein a change in activity relative to a reference value is an indication that the test compound is an agent useful in a disorder related to sodium current. In one embodiment, the disorder is pain, paraesthesia, stroke, head trauma, a neurodegenerative disorder, or a disorder related to hyperexcitability of neurons.

The method can further include administering the compound in vivo (e.g., using an animal model). The method can further include modifying the compound for use in vivo. The method can further include evaluating modulation of a related human Na<sub>v</sub>1.3  $\alpha$  subunit polypeptide by the compound.

In another aspect, the invention features a method for treating a subject having a disorder related to sodium channel current, the method comprising: identifying an agent that selectively binds a mNa<sub>v</sub>1.3  $\alpha$  subunit polypeptide, and administering to a subject in need of such treatment a pharmacological agent which is selective for a sodium channel comprising a mNa<sub>v</sub>1.3  $\alpha$  subunit polypeptide.

In one embodiment, the disorder is pain, paraesthesia, stroke, head trauma, a neurodegenerative disorder, or a disorder related to hyperexcitability of neurons.

25

#### BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 depicts an alignment of the amino acid sequences of a novel murine Na<sub>v</sub>1.3  $\alpha$  subunit amino acid sequence with two human Na<sub>v</sub>1.3  $\alpha$  subunit sequences and a rat Na<sub>v</sub>1.3  $\alpha$  subunit sequence.

FIG. 2 depicts an alignment of the amino acid sequences of a novel murine  $\text{Na}_v1.3 \alpha$  subunit amino acid sequence with an amino acid sequence encoded by a partial nucleotide sequence found under GenBank® Acc. No. NM\_018732.

5 FIG. 3 is a graph depicting sodium currents in *Xenopus* oocytes transfected with a novel murine  $\text{Na}_v1.3 \alpha$  subunit, and depolarized at a range of voltages.

FIG. 4 is a graph depicting the average current/voltage relationship in *Xenopus* oocytes transfected with a novel murine  $\text{Na}_v1.3 \alpha$  subunit, which were depolarized at a range of voltages.

#### DETAILED DESCRIPTION

The invention is based, in part, on the identification of a novel  $\text{Na}^+$  channel type III  $\alpha$  10 ( $\text{Na}_v1.3$ ) subunit. The novel cDNA was isolated from murine brain tissue. The coding sequence of the cDNA (SEQ ID NO:1) corresponds to nucleotide 8 through nucleotide 5947 of the sequence shown in Table 1. The predicted amino acid sequence of the novel subunit is shown in SEQ ID NO:2. Related human and rat subunit nucleotide sequences are found in GenBank® under accession numbers NM\_006922, AF225986, and NM\_013119 (SEQ ID NO:4, SEQ ID 15 NO:6, and SEQ ID NO:8). A fragment of a related murine  $\text{Na}^+$  channel cDNA has been cloned and is found under GenBank® accession no. NM\_018732 (SEQ ID NO:10). An alignment of SEQ ID NO:2 with this sequence is shown in FIG. 2.

The novel murine nucleic acid sequence contains differences as compared to the related 20 human and rat sequences. These differences are predicted to encode a unique polypeptide sequence. An alignment of the predicted amino acid sequence of the novel mouse  $\text{Na}^+$  channel  $\alpha$  subunit and related rat and human subunits is depicted in Figure 1.

#### Voltage-gated $\text{Na}^+$ Channels

Voltage-gated  $\text{Na}^+$  channels are multisubunit transmembrane proteins having an  $\alpha$  25 subunit of approximately 260 kilodaltons (kD), a  $\beta 1$  subunit of approximately 35 kD, and a  $\beta 2$  subunit of approximately 35 kD. These channels mediate the influx of sodium ions into cells. The  $\alpha$  subunit forms the voltage-sensitive, pore-forming part of the channel, and the  $\beta$  subunits regulate gating of the  $\alpha$  subunit and cell-cell interactions. The  $\alpha$  subunit of  $\text{Na}^+$  channels contains four repeated domains (I-IV). Each of these repeated domains contains six transmembrane segments (S1-S6) (Reviewed in Baker, MD, and Wood, JN. *Trends in Pharm Sci.* 22(1):27-31, 2001).

Voltage-gated  $\text{Na}^+$  channels undergo cycles of resting (polarized; closed channel; activateable), open (depolarized; open channel; activated) and closed (depolarized, closed channel; inactivated) states in response to changes in membrane polarization. Most voltage-gated  $\text{Na}^+$  channels close rapidly (i.e., within milliseconds) of opening. Charged regions of the ion-conducting  $\alpha$  subunit are sensitive to changes in membrane polarization.

$\text{Na}^+$  channels enable production and propagation of electrical impulses in excitable cells. For example,  $\text{Na}^+$  channels expressed in primary sensory neurons (e.g., dorsal root ganglion (DRG) neurons, trigeminal neurons) produce a depolarizing upstroke in response to stimulation, which in turn is required for transmission of sensory information.

Subtypes of  $\text{Na}^+$  channels can be distinguished based on their sensitivity to inhibition by tetrodotoxin (TTX), a guanidinium toxin expressed by Puffer fish. TTX blocks the activity of  $\text{Na}_v1.1$ ,  $\text{Na}_v1.2$ , and  $\text{Na}_v1.3$   $\alpha$  subunit channels at concentrations in the nanomolar range. All three types of channels are expressed in brain. Disregulation of levels of  $\text{Na}^+$  channels in response to injury can lead to hyperexcitability of neuronal  $\text{Na}^+$  channels. This type of disregulation is thought to contribute to chronic pain. For example, axonal transection causes downregulation of certain  $\text{Na}^+$  channels and upregulation of  $\text{Na}_v1.3$   $\alpha$  subunit channels (reviewed in Waxman, S.G., *Nature Rev.* 2:652-659, 2001), which leads to inappropriate repetitive firing of neurons.

As used herein, a "sodium channel  $\alpha$  subunit" or " $\text{Na}^+$  channel  $\alpha$  subunit" refers to a protein which is involved in receiving, conducting, and transmitting signals, in a cell, such as a neuronal cell, e.g., a dorsal root ganglion. "Murine  $\text{Na}_v1.3$   $\alpha$  subunit" or "m $\text{Na}_v1.3$   $\alpha$  subunit" refers to a murine type III  $\alpha$  subunit described herein.

As used herein, a " $\text{Na}^+$  channel mediated activity" refers to an activity, function, or response that involves a  $\text{Na}^+$  channel, e.g., a  $\text{Na}^+$  channel in a brain cell or a muscle cell.  $\text{Na}^+$  channel mediated activities are activities involved in receiving, conducting, and transmitting signals in, for example, the nervous system (e.g., the central nervous system and the peripheral nervous system), muscle tissue, cardiac tissue, and other cells and tissues.  $\text{Na}^+$  channel mediated activities include, for example, regulation of  $\text{Na}^+$  influx into cells and transmission of sensory stimuli. Also, as used interchangeably herein a " $\text{Na}^+$  channel  $\alpha$  subunit activity", "m $\text{Na}_v1.3$  channel activity", "biological activity of  $\text{Na}^+$  channel  $\alpha$  subunit" or "functional activity of a  $\text{Na}^+$

channel  $\alpha$  subunit", refers to an activity, function, or response of a  $\text{Na}^+$  channel  $\alpha$  subunit protein, polypeptide or nucleic acid molecule.

Isolated proteins of the present invention, e.g., m $\text{Na}_v1.3$   $\alpha$  subunit proteins described herein, have an amino acid sequence sufficiently homologous to the amino acid sequence of SEQ ID NO:2 or are encoded by a nucleotide sequence sufficiently homologous to SEQ ID NO:1. As used herein, the term "sufficiently homologous" refers to a first amino acid or nucleotide sequence which contains a sufficient or minimum number of identical or equivalent (e.g., an amino acid residue-which has a similar side chain) amino acid residues or nucleotides to a second amino acid or nucleotide sequence such that the first and second amino acid or nucleotide sequences share common structural domains or motifs or a common functional activity.

Accordingly, another embodiment of the invention features isolated m $\text{Na}_v1.3$   $\alpha$  subunit proteins and polypeptides, fragments, and variants thereof having m $\text{Na}_v1.3$   $\alpha$  subunit activity.

The novel m $\text{Na}_v1.3$   $\alpha$  subunit coding sequence (SEQ ID NO:1), which is approximately 5940 nucleotides in length, encodes a protein which is approximately 1980 amino acid residues in length. The gene encoding this novel subunit is expressed in the brain.  $\text{Na}_v1.3$  channels are expressed, e.g., in brain, heart, and skeletal muscle. An  $\text{Na}_v1.3$  channel can be expressed at low or high levels in a given tissue. Some  $\text{Na}_v1.3$  channels expressed in neurons are upregulated following neuronal injury (Waxman, SG, et al. *Brain Res.* 8886:5-14, 2000; Kim, CH, et al. *Mol. Brain Res.* 95:153-161, 2001).

Various aspects of the invention are described in further detail in the following subsections.

#### *Isolated Nucleic Acid Molecules*

One aspect of the invention pertains to isolated nucleic acid molecules that encode m $\text{Na}_v1.3$   $\alpha$  subunit polypeptides or biologically active portions thereof, as well as nucleic acid fragments sufficient for use as hybridization probes to identify nucleic acid molecules that encode related isoforms of the m $\text{Na}_v1.3$   $\alpha$  subunit described herein and fragments for use as PCR primers for the amplification or mutation of m $\text{Na}_v1.3$   $\alpha$  subunit nucleic acid molecules. As used herein, the term "nucleic acid molecule" is intended to include DNA molecules (e.g., cDNA or genomic DNA) and RNA molecules (e.g., mRNA) and analogs of the DNA or RNA generated

using nucleotide analogs. The nucleic acid molecule can be single-stranded or double-stranded, but preferably is double-stranded DNA.

An "isolated" nucleic acid molecule is one that is separated from other nucleic acid molecules which are present in the natural source of the nucleic acid. An "isolated" nucleic acid is free of sequences that naturally flank the nucleic acid (i.e., sequences located at the 5' and 3' ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, the isolated mNa<sub>v</sub>1.3  $\alpha$  subunit nucleic acid molecule can contain less than about 5 kb, 4 kb, 3 kb, 2kb, 1 kb, 0.5 kb or 0.1 kb of nucleotide sequences which naturally flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid is derived. Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, can be substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized.

A nucleic acid molecule of the present invention, e.g., a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:1, or a portion thereof, can be isolated using standard molecular biology techniques and the sequence information provided herein. Using all or portion of the nucleic acid sequence of SEQ ID NO:1 as a hybridization probe, mNa<sub>v</sub>1.3  $\alpha$  subunit nucleic acid molecules can be isolated using standard hybridization and cloning techniques (e.g., as described in Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual*. 2nd, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989).

Moreover, a nucleic acid molecule encompassing all or a portion of SEQ ID NO:1 can be isolated by the polymerase chain reaction (PCR) using synthetic oligonucleotide primers designed based upon the sequence of SEQ ID NO:1.

A nucleic acid of the invention can be amplified using cDNA, mRNA or alternatively, genomic DNA, as a template and appropriate oligonucleotide primers according to standard PCR amplification techniques. The nucleic acid so amplified can be cloned into an appropriate vector and characterized by DNA sequence analysis. Furthermore, oligonucleotides corresponding to Na<sup>+</sup> channel  $\alpha$  subunit nucleotide sequences can be prepared by standard synthetic techniques, e.g., using an automated DNA synthesizer.

In another embodiment, an isolated nucleic acid molecule of the invention comprises a mNa<sub>v</sub>1.3  $\alpha$  subunit nucleic acid molecule which is a complement of the nucleotide sequence shown in SEQ ID NO:1, or a portion of these nucleotide sequences. A nucleic acid molecule which is complementary to the nucleotide sequence shown in SEQ ID NO:1 is one which is sufficiently complementary to the nucleotide sequence shown in SEQ ID NO:1 such that it can hybridize to the nucleotide sequence shown in SEQ ID NO:1.

In still another preferred embodiment, an isolated nucleic acid molecule of the present invention comprises a nucleotide sequence which is at least about 75%, 85%, 95% or more homologous to the entire length of the nucleotide sequence shown in SEQ ID NO:1, or a portion of this nucleotide sequence.

Moreover, the nucleic acid molecule of the invention can comprise only a portion of the nucleic acid sequence of SEQ ID NO:1, for example a fragment which can be used as a probe or primer or a fragment encoding a biologically active portion of a mNa<sub>v</sub>1.3  $\alpha$  subunit protein. The nucleotide sequence determined from the cloning of the mNa<sub>v</sub>1.3  $\alpha$  subunit cDNA allows for the generation of probes and primers designed for use in identifying and/or cloning related isoforms, as well as homologues from other species. The probe/primer typically comprises substantially purified oligonucleotide. The oligonucleotide typically comprises a region of nucleotide sequence that hybridizes under stringent conditions to at least about 12 or 15, preferably about 20 or 25, more preferably about 30, 35, 40, 45, 50, 55, 60, 65, or 75 consecutive nucleotides of a sense sequence of SEQ ID NO:1, of an anti-sense sequence of SEQ ID NO:1, or of a naturally occurring allelic variant or mutant of SEQ ID NO:1.

Probes based on the mNa<sub>v</sub>1.3  $\alpha$  subunit nucleotide sequences can be used to detect transcripts encoding related isoforms. The probe can further include a label group attached thereto, e.g., the label group can be a radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor. Such probes can be used as a part of a diagnostic test kit for identifying cells or tissue which express or misexpress a mNa<sub>v</sub>1.3  $\alpha$  subunit protein, such as by measuring a level of a mNa<sub>v</sub>1.3  $\alpha$  subunit-encoding nucleic acid in a sample of cells from a subject e.g., detecting mNa<sub>v</sub>1.3  $\alpha$  subunit mRNA levels or determining whether a genomic mNa<sub>v</sub>1.3  $\alpha$  subunit gene has been mutated or deleted in a region that would affect expression of the mNa<sub>v</sub>1.3  $\alpha$  subunit isoform.

5 A nucleic acid fragment encoding a "biologically active portion of a mNa<sub>v</sub>1.3  $\alpha$  subunit protein" can be prepared by isolating a portion of the nucleotide sequence of SEQ ID NO:1, which encodes a polypeptide having a mNa<sub>v</sub>1.3  $\alpha$  subunit biological activity (the biological activities of the mNa<sub>v</sub>1.3  $\alpha$  subunit proteins are described herein), expressing the encoded portion of the mNa<sub>v</sub>1.3  $\alpha$  subunit protein (e.g., by recombinant expression in vitro) and assessing the activity of the encoded portion of the mNa<sub>v</sub>1.3  $\alpha$  subunit protein.

10 The invention further encompasses nucleic acid molecules that differ from the nucleotide sequence shown in SEQ ID NO:1, due to degeneracy of the genetic code and thus encode the same mNa<sub>v</sub>1.3  $\alpha$  subunit proteins as those encoded by the nucleotide sequence shown in SEQ ID NO:1. In another embodiment, an isolated nucleic acid molecule of the invention has a 15 nucleotide sequence encoding a protein having an amino acid sequence shown in SEQ ID NO:2.

15 In addition to the mNa<sub>v</sub>1.3  $\alpha$  subunit nucleotide sequences shown in SEQ ID NO:1, it will be appreciated by those skilled in the art that DNA sequence polymorphisms that lead to changes in the amino acid sequences of the mNa<sub>v</sub>1.3  $\alpha$  subunit proteins may exist within a 20 population. Such genetic polymorphism in the mNa<sub>v</sub>1.3  $\alpha$  subunit genes may exist among individuals within a population due to natural allelic variation. As used herein, the terms "gene" and "recombinant gene" refer to nucleic acid molecules comprising an open reading frame encoding a mNa<sub>v</sub>1.3  $\alpha$  subunit protein. Such natural allelic variations can typically result in 1-5% variance in the nucleotide sequence of a mNa<sub>v</sub>1.3  $\alpha$  subunit gene. Any and all such 25 nucleotide variations and resulting amino acid polymorphisms in mNa<sub>v</sub>1.3  $\alpha$  subunit genes that are the result of natural allelic variation and that do not alter the functional activity of a mNa<sub>v</sub>1.3  $\alpha$  subunit protein are intended to be within the scope of the invention.

25 Moreover, nucleic acid molecules encoding other mNa<sub>v</sub>1.3  $\alpha$  subunit channel family members and thus which have a nucleotide sequence which differs from the mNa<sub>v</sub>1.3  $\alpha$  subunit sequences of SEQ ID NO:1 are intended to be within the scope of the invention. For example, another mNa<sub>v</sub>1.3  $\alpha$  subunit cDNA can be identified based on the nucleotide sequence of a mNa<sub>v</sub>1.3  $\alpha$  subunit (e.g., SEQ ID NO:1). Moreover, nucleic acid molecules encoding mNa<sub>v</sub>1.3  $\alpha$  subunit proteins from different species, and thus which have a nucleotide sequence which 30 differs from the mNa<sub>v</sub>1.3  $\alpha$  subunit sequences of SEQ ID NO:1 are intended to be within the scope of the invention.

Nucleic acid molecules corresponding to natural allelic variants and homologues of the mNa<sub>v</sub>1.3  $\alpha$  subunit cDNAs of the invention can be isolated based on their homology to the mNa<sub>v</sub>1.3  $\alpha$  subunit nucleic acids disclosed herein using the cDNAs disclosed herein, or a portion thereof, as a hybridization probe according to standard hybridization techniques under stringent hybridization conditions.

Accordingly, in another embodiment, an isolated nucleic acid molecule of the invention is at least 15, 20, 25, 30 or more nucleotides in length and hybridizes under stringent conditions to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1. Preferably, the molecule hybridizes under highly stringent conditions. In other embodiments, the nucleic acid is at least 30, 300, 500, 700, 850, 950, or 2000 nucleotides in length. As used herein, the term "hybridizes under stringent conditions" is intended to describe conditions for hybridization and washing under which nucleotide sequences at least 60%, 85%, or 95% homologous to each other typically remain hybridized to each other. Hybridization conditions are known to those skilled in the art and can be found in Current Protocols in Molecular Biology, John Wiley & Sons, N.Y., 6.3  $\alpha$  subunit.1-6.3  $\alpha$  subunit.6, 1991. Moderate hybridization conditions are defined as equivalent to hybridization in 2X sodium chloride/sodium citrate (SSC) at 30°C, followed by a wash in 1 X SSC, 0.1% SDS at 50°C. Highly stringent conditions are defined as equivalent to hybridization in 6X sodium chloride/sodium citrate (SSC) at 45°C, followed by a wash in 0.2 X SSC, 0.1% SDS at 65°C.

An isolated nucleic acid molecule of the invention that hybridizes under moderate or highly stringent conditions to the sequence of SEQ ID NO:1 can correspond to a naturally-occurring nucleic acid molecule. As used herein, a "naturally-occurring" nucleic acid molecule refers to an RNA or DNA molecule having a nucleotide sequence that occurs in nature (e.g., encodes a natural protein).

In addition to naturally-occurring allelic variants of the mNa<sub>v</sub>1.3  $\alpha$  subunit sequences that may exist in the population, the skilled artisan will further appreciate that changes can be introduced by mutation into the nucleotide sequences of SEQ ID NO:1, thereby leading to changes in the amino acid sequence of the encoded mNa<sub>v</sub>1.3  $\alpha$  subunit proteins, without altering the functional ability of the mNa<sub>v</sub>1.3  $\alpha$  subunit proteins. For example, nucleotide substitutions leading to amino acid substitutions at "non-essential" amino acid residues can be made in the sequence of SEQ ID NO:1. A "non-essential" amino acid residue is a residue that can be altered

from the wild-type sequence of mNa<sub>v</sub>1.3  $\alpha$  subunit without altering the biological activity, whereas an "essential" amino acid residue is required for biological activity. For example, amino acid residues that are conserved among the mNa<sub>v</sub>1.3  $\alpha$  subunit proteins of the present invention, are predicted to be particularly unamenable to alteration. Furthermore, additional amino acid 5 residues that are conserved between the mNa<sub>v</sub>1.3  $\alpha$  subunit proteins of the present invention and other mNa<sub>v</sub>1.3  $\alpha$  subunit channel subunits are not likely to be amenable to alteration.

Accordingly, another aspect of the invention pertains to nucleic acid molecules encoding mNa<sub>v</sub>1.3  $\alpha$  subunit proteins that contain changes in amino acid residues that are not essential for activity. Such mNa<sub>v</sub>1.3  $\alpha$  subunit proteins differ in amino acid sequence from SEQ ID NO:2, yet 10 retain biological activity. Biological activity can be measured by an assay described herein, e.g., a Na<sup>+</sup> channel activity assay, e.g., a Na<sup>+</sup> influx assay. In one embodiment, the isolated nucleic acid molecule comprises a nucleotide sequence encoding a protein, wherein the protein comprises an amino acid sequence at least about 65%, 75%, 85%, 95% or more homologous to SEQ ID NO:2.

15 An isolated nucleic acid molecule encoding a mNa<sub>v</sub>1.3  $\alpha$  subunit protein homologous to the protein of SEQ ID NO:2 can be created by introducing one or more nucleotide substitutions, additions or deletions into the nucleotide sequence of SEQ ID NO:1 such that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein. Mutations can be introduced into SEQ ID NO:1 by standard techniques, such as site-directed mutagenesis and 20 PCR-mediated mutagenesis. Conservative amino acid substitutions can be made at one or more predicted non-essential amino acid residues. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These 25 families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted nonessential amino acid residue in a mNa<sub>v</sub>1.3  $\alpha$  30 subunit protein is preferably replaced with another amino acid residue from the same side chain

family. Following mutagenesis of SEQ ID NO:1, the encoded protein can be expressed and the activity of the protein can be determined.

A mutant mNa<sub>v</sub>1.3  $\alpha$  subunit protein can be assayed for the ability to (1) interact with a non mNa<sub>v</sub>1.3  $\alpha$  subunit protein molecule, e.g., Na<sup>+</sup> channel  $\beta$ 1 or  $\beta$ 2 subunits, or TTX; or (2) 5 modulate membrane excitability.

In addition to the nucleic acid molecules encoding mNa<sub>v</sub>1.3  $\alpha$  subunit proteins described above, another aspect of the invention pertains to isolated nucleic acid molecules that are antisense thereto. An "antisense" nucleic acid comprises a nucleotide sequence that is complementary to a "sense" nucleic acid encoding a protein, e.g., complementary to the coding 10 strand of a double-stranded cDNA molecule or complementary to an mRNA sequence.

Accordingly, an antisense nucleic acid can hydrogen bond to a sense nucleic acid. The antisense nucleic acid can be complementary to an entire mNa<sub>v</sub>1.3  $\alpha$  subunit coding strand, or to only a portion thereof. In one embodiment, an antisense nucleic acid molecule is antisense to a "coding 15 region" of the coding strand of a nucleotide sequence encoding mNa<sub>v</sub>1.3  $\alpha$  subunit. The term "coding region" refers to the region of the nucleotide sequence comprising codons which are 20 translated into amino acid residues (e.g., the coding region of a mna<sub>v</sub>1.3  $\alpha$  subunit corresponds to SEQ ID NO:1). In another embodiment, the antisense nucleic acid molecule is antisense to a "noncoding region" of the coding strand of a nucleotide sequence encoding a mNa<sub>v</sub>1.3  $\alpha$  subunit. The term "noncoding region" refers to 5' and 3' sequences that flank the coding region that are not translated into amino acids (i.e., also referred to as 5' and 3' untranslated regions).

Given the coding strand sequences encoding a mNa<sub>v</sub>1.3  $\alpha$  subunit disclosed herein (e.g., SEQ ID NO:1), antisense nucleic acids of the invention can be designed according to the rules of Watson and Crick base pairing. The antisense nucleic acid molecule can be complementary to the entire coding region of mNa<sub>v</sub>1.3  $\alpha$  subunit mRNA, but more preferably is an oligonucleotide 25 which is antisense to only a portion of the coding or noncoding region of mNa<sub>v</sub>1.3  $\alpha$  subunit mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site of a mNa<sub>v</sub>1.3  $\alpha$  subunit mRNA. An antisense oligonucleotide can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 nucleotides in length. An antisense nucleic acid of the invention can be constructed using chemical synthesis 30 and enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (e.g., an antisense oligonucleotide) can be chemically synthesized using naturally

occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, e.g., phosphorothioate derivatives and acridine substituted nucleotides can be used. Examples of modified nucleotides which can be used to generate the antisense nucleic acid include 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, 5 hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil 1,5-carboxymethylaminoethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, 10 N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxymethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiacytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-15 carboxypropyl) uracil, and 2,6-diaminopurine. Alternatively, the antisense nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (i.e., RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest, described further in the following subsection).

20 The antisense nucleic acid molecules of the invention are typically administered to a subject or generated in situ such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding a mNa<sub>v</sub>1.3  $\alpha$  subunit protein to thereby inhibit expression of the protein, e.g., by inhibiting transcription and/or translation. The hybridization can be by conventional nucleotide complementarity to form a stable duplex, or, for example, in the case of an antisense 25 nucleic acid molecule which binds to DNA duplexes, through specific interactions in the major groove of the double helix. An example of a route of administration of antisense nucleic acid molecules of the invention include direct injection at a tissue site. Alternatively, antisense nucleic acid molecules can be modified to target selected cells and then administered systemically. For example, for systemic administration, antisense molecules can be modified 30 such that they specifically bind to receptors or antigens expressed on a selected cell surface, e.g., by linking the antisense nucleic acid molecules to peptides or antibodies that bind to cell surface

receptors or antigens. The antisense nucleic acid molecules can also be delivered to cells using the vectors described herein. To achieve sufficient intracellular concentrations of the antisense molecules, vector constructs in which the antisense nucleic acid molecule is placed under the control of a strong pol II or pol III promoter are preferred.

5 In yet another embodiment, the antisense nucleic acid molecule of the invention is an  $\alpha$ -anomeric nucleic acid molecule. An  $\alpha$ -anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual  $\beta$ -units, the strands run parallel to each other (Gaultier et al. *Nucleic Acids. Res.* 15:6625-6641, 1987). The antisense nucleic acid molecule can also comprise a 2'-o-methylribonucleotide (Inoue et al. (1987) *Nucleic Acids Res.* 15:6131-6148) or a chimeric RNA-DNA analogue (Inoue et al. *FEBS Lett.* 215:327-330, 1987).

10

In still another embodiment, an antisense nucleic acid of the invention is a ribozyme. Ribozymes are catalytic RNA molecules with ribonuclease activity which are capable of cleaving a single-stranded nucleic acid, such as an mRNA, to which they have a complementary 15 region. Thus, ribozymes (e.g., hammerhead ribozymes (described in Haselhoff and Gerlach *Nature* 334:585-591, 1988)) can be used to catalytically cleave mNa<sub>v</sub>1.3  $\alpha$  subunit mRNA transcripts to thereby inhibit translation of mNa<sub>v</sub>1.3  $\alpha$  subunit mRNA. A ribozyme having specificity for a mNa<sub>v</sub>1.3  $\alpha$  subunit-encoding nucleic acid can be designed based upon the nucleotide sequence of a mNa<sub>v</sub>1.3  $\alpha$  subunit cDNA disclosed herein (i.e., SEQ ID NO:1). For 20 example, a derivative of a Tetrahymena L-19 IVS RNA can be constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in a mNa<sub>v</sub>1.3  $\alpha$  subunit-encoding mRNA. See, e.g., Cech et al. U.S. Pat. No. 4,987,071; and Cech et al. U.S. Pat. No. 5,116,742. Alternatively, mNa<sub>v</sub>1.3  $\alpha$  subunit mRNA can be used to 25 select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. See, e.g., Bartel, D. and Szostak, J. W. *Science* 261:1411-1418, 1993.

In another embodiment of the present invention, RNA interference (RNAi) can be used to inhibit the expression of a mNa<sub>v</sub>1.3  $\alpha$  subunit protein. Various inhibitory RNAi molecules can be identified and those that inhibit expression of a mNa<sub>v</sub>1.3  $\alpha$  subunit can be formulated as pharmaceutical compositions to be administered in the methods of treatment described herein.

30 RNAi is a term used to refer to the mechanism by which a particular mRNA is degraded in host cells. To inhibit an mRNA, double-stranded RNA (dsRNA) corresponding to a portion of

the gene to be silenced (e.g., a gene encoding a mNa<sub>v</sub>1.3  $\alpha$  subunit polypeptide) is introduced into a cell. The dsRNA is digested into 21-25 nucleotide-long duplexes called short interfering RNAs (or siRNAs), which bind to a nuclease complex to form what is known as the RNA-induced silencing complex (or RISC). The RISC targets the homologous transcript by base pairing interactions between one of the siRNA strands and the endogenous mRNA. It then cleaves the mRNA about 12 nucleotides from the 3' terminus of the siRNA (see Sharp *et al.*, *Genes Dev.* 15:485-490, 2001, and Hammond *et al.*, *Nature Rev. Gen.* 2:110-119, 2001). RNAi has proven successful in human cells, including human embryonic kidney and HeLa cells (see, e.g., Elbashir *et al.*, *Nature* 411:494-498, 2001). Gene silencing can be induced in mammalian cells by enforcing endogenous expression of RNA hairpins (see Paddison *et al.*, *Proc. Natl. Acad. Sci. USA* 99:1443-1448, 2002) or by transfection of small (21-23 nt) dsRNA (reviewed in Caplen, *Trends in Biotech.* 20:49-51, 2002).

RNAi technology utilizes standard molecular biology methods. The dsRNA (which, here, for example, would correspond to the sequence encoding a mNa<sub>v</sub>1.3  $\alpha$  subunit polypeptide) can be produced by standard methods (e.g., by simultaneously transcribing both strands of a template DNA corresponding to a mNa<sub>v</sub>1.3  $\alpha$  subunit sequence with T7 RNA polymerase; the RNA can also be chemically synthesized or recombinantly produced). Kits for producing dsRNA are available commercially (from, e.g., New England Biolabs, Inc). The RNA used to mediate RNAi can include synthetic or modified nucleotides, such as phosphorothioate nucleotides. Methods of transfecting cells with dsRNA or with plasmids engineered to make dsRNA are also routine in the art.

Gene silencing effects similar to those observed with RNAi have been reported in mammalian cells transfected with an mRNA-cDNA hybrid construct (Lin *et al.*, *Biochem. Biophys. Res. Comm.* 281:639-644, 2001). Accordingly, mRNA-cDNA hybrids containing mNa<sub>v</sub>1.3  $\alpha$  subunit sequence, as well as duplexes that contain mNa<sub>v</sub>1.3  $\alpha$  subunit sequence (e.g., duplexes containing 21-23 bp monomers), are within the scope of the present invention. The hybrids and duplexes can be tested for activity according to the assays described herein (*i.e.*, they can serve as the test agents), and those that exhibit inhibitory activity can be used to treat patients who have, or who may develop, a disease or condition associated with a mNa<sub>v</sub>1.3  $\alpha$  subunit activity, e.g., neuropathic pain.

The dsRNA molecules of the invention (double-stranded RNA molecules corresponding to portions of a mNa<sub>1.3</sub>  $\alpha$  subunit gene) can vary in a number of ways. For example, they can include a 3' hydroxyl group and, as noted above, can contain strands of 21, 22, or 23 consecutive nucleotides. Moreover, they can be blunt ended or include an overhanging end at either the 3' 5 end, the 5' end, or both ends. For example, at least one strand of the RNA molecule can have a 3' overhang from about 1 to about 6 nucleotides (e.g., 1-5, 1-3, 2-4 or 3-5 nucleotides (whether pyrimidine or purine nucleotides) in length. Where both strands include an overhang, the length of the overhangs may be the same or different for each strand. To further enhance the stability of the RNA duplexes, the 3' overhangs can be stabilized against degradation (by, e.g., including 10 purine nucleotides, such as adenosine or guanosine nucleotides or replacing pyrimidine nucleotides by modified analogues (e.g., substitution of uridine 2 nucleotide 3' overhangs by 2'-deoxythymidine is tolerated and does not affect the efficiency of RNAi). The single stranded mNa<sub>1.3</sub>  $\alpha$  subunit RNA molecules that make up the duplex or hybrid inhibitor, or that act 15 simply as antisense RNA oligonucleotides, are also within the scope of the invention. Any dsRNA can be used in the methods of the present invention, provided it has sufficient homology to a target gene of interest, e.g., a mNa<sub>1.3</sub>  $\alpha$  subunit gene, to mediate RNAi. While duplexes having 21-23 nucleotides are described above, the invention is not so limited; there is no upper limit on the length of the dsRNA that can be used (e.g., the dsRNA can range from about 21 base 20 pairs of the gene to the full length of the gene or more (e.g., 50-100, 100-250, 250-500, 500- 1000, or over 1000 base pairs).

When these nucleic acids are administered to a human, they can reduce mNa<sub>1.3</sub>  $\alpha$  subunit mRNA levels, thereby inhibiting expression of a mNa<sub>1.3</sub>  $\alpha$  subunit. The cell or organism is maintained under conditions in which mNa<sub>1.3</sub>  $\alpha$  subunit mRNA is degraded, thereby mediating RNAi in the cell or organism. Alternatively, cells can be obtained from the 25 individual, treated *ex vivo*, and re-introduced into the individual.

In yet another embodiment, the mNa<sub>1.3</sub>  $\alpha$  subunit nucleic acid molecules of the present invention can be modified at the base moiety, sugar moiety or phosphate backbone to improve, e.g., the stability, hybridization, or solubility of the molecule. For example, the deoxyribose phosphate backbone of the nucleic acid molecules can be modified to generate peptide nucleic acids (see Hyrup B. et al. *Bioorganic & Medicinal Chemistry* 4 (1): 5-23, 1996). As used herein, the terms "peptide nucleic acids" or "PNAs" refer to nucleic acid mimics, e.g., DNA 30

mimics, in which the deoxyribose phosphate backbone is replaced by a pseudopeptide backbone and only the four natural nucleobases are retained. The neutral backbone of PNAs has been shown to allow for specific hybridization to DNA and RNA under conditions of low ionic strength. The synthesis of PNA oligomers can be performed using standard solid phase peptide synthesis protocols as described in Hyrup B. et al. *supra*; Perry-O'Keefe et al. *Proc. Natl. Acad. Sci.* 93: 14670-675, 1996.

PNAs of mNa<sub>v</sub>1.3  $\alpha$  subunit nucleic acid molecules can be used in therapeutic and diagnostic applications. For example, PNAs can be used as antisense or antigenic agents for sequence-specific modulation of gene expression by, for example, inducing transcription or 10 translation arrest or inhibiting replication. PNAs of mNa<sub>v</sub>1.3  $\alpha$  subunit nucleic acid molecules can also be used in the analysis of single base pair mutations in a gene, (e.g., by PNA-directed PCR clamping); as 'artificial restriction enzymes' when used in combination with other enzymes, (e.g., S1 nucleases (Hyrup B. , *supra*)); or as probes or primers for DNA sequencing or hybridization (Hyrup B. et al., *supra*; Perry-O'Keefe, *supra*).

15 In another embodiment, PNAs of mNa<sub>v</sub>1.3  $\alpha$  subunit can be modified, (e.g., to enhance their stability or cellular uptake), by attaching lipophilic or other helper groups to PNA, by the formation of PNA-DNA chimeras, or by the use of liposomes or other techniques of drug delivery known in the art.

20 In other embodiments, the oligonucleotide may include other appended groups such as peptides (e.g., for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (see, e.g., Letsinger et al. *Proc. Natl. Acad. Sci. US.* 86:6553-6556, 1989; Lemaitre et al. *Proc. Natl. Acad. Sci. USA* 84:648-652, 1987; PCT Publication No. WO88/09810) or the blood-brain barrier (see, e.g., PCT Publication No. WO89/10134). In addition, oligonucleotides can be modified with hybridization-triggered cleavage agents (See, e.g., Krol et al. *Bio-Techniques* 6:958-976, 1988) or intercalating agents (See, e.g., Zon *Pharm. Res.* 5:539-549, 1988). To this end, the oligonucleotide may be conjugated to another molecule, (e.g., a peptide, hybridization triggered cross-linking agent, transport agent, or hybridization-triggered cleavage agent).

30 *Isolated mNa<sub>v</sub>1.3 Proteins and Anti-mNa<sub>v</sub>1.3 Antibodies*

One aspect of the invention pertains to isolated mNa<sub>v</sub>1.3  $\alpha$  subunit proteins, and biologically active portions thereof, as well as polypeptide fragments suitable for use as immunogens to raise anti-mNa<sub>v</sub>1.3  $\alpha$  subunit antibodies. In one embodiment, native mNa<sub>v</sub>1.3  $\alpha$  subunit proteins can be isolated from cells or tissue sources by an appropriate purification scheme using standard protein purification techniques. In another embodiment, mNa<sub>v</sub>1.3  $\alpha$  subunit proteins are produced by recombinant DNA techniques. Alternative to recombinant expression, a mNa<sub>v</sub>1.3  $\alpha$  subunit protein or polypeptide can be synthesized chemically using standard peptide synthesis techniques.

An "isolated" or "purified" protein or biologically active portion thereof is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the mNa<sub>v</sub>1.3  $\alpha$  subunit protein is derived, or substantially free from chemical precursors or other chemicals when chemically synthesized. The language "substantially free of cellular material" includes preparations of mNa<sub>v</sub>1.3  $\alpha$  subunit protein in which the protein is separated from cellular components of the cells from which it is isolated or recombinantly produced. In one embodiment, the language "substantially free of cellular material" includes preparations of mNa<sub>v</sub>1.3  $\alpha$  subunit protein having less than about 30% (by dry weight) of non-mNa<sub>v</sub>1.3  $\alpha$  subunit protein (also referred to herein as a "contaminating protein"), more preferably less than about 20%, 10%, or 5% of non-mNa<sub>v</sub>1.3  $\alpha$  subunit protein. When the mNa<sub>v</sub>1.3  $\alpha$  subunit protein or biologically active portion thereof is recombinantly produced, it is also preferably substantially free of culture medium, i.e., culture medium represents less than about 20%, 10%, or 5% of the volume of the protein preparation.

The language "substantially free of chemical precursors or other chemicals" includes preparations of mNa<sub>v</sub>1.3  $\alpha$  subunit protein in which the protein is separated from chemical precursors or other chemicals that are involved in the synthesis of the protein. In one embodiment, the language "substantially free of chemical precursors or other chemicals" includes preparations of mNa<sub>v</sub>1.3  $\alpha$  subunit protein having less than about 30%, 20%, 10%, or 5% (by dry weight) of chemical precursors or non-mNa<sub>v</sub>1.3  $\alpha$  subunit chemicals.

As used herein, a "biologically active portion" of a mNa<sub>v</sub>1.3  $\alpha$  subunit protein includes a fragment of a mNa<sub>v</sub>1.3  $\alpha$  subunit protein which participates in an interaction between a mNa<sub>v</sub>1.3  $\alpha$  subunit molecule and a non mNa<sub>v</sub>1.3  $\alpha$  subunit molecule. Biologically active portions of a

mNa<sub>v</sub>1.3  $\alpha$  subunit proteins include peptides comprising amino acid sequences sufficiently homologous to or derived from the amino acid sequence of the mNa<sub>v</sub>1.3  $\alpha$  subunit protein, e.g., the amino acid sequence shown in SEQ ID NO:2, which include less amino acids than the full length mNa<sub>v</sub>1.3  $\alpha$  subunit proteins, and exhibit at least one activity of a mNa<sub>v</sub>1.3  $\alpha$  subunit protein. Typically, biologically active portions comprise a domain or motif with at least one activity of the mNa<sub>v</sub>1.3  $\alpha$  subunit protein, e.g., binding of a  $\beta$ 1 or  $\beta$ 2 Na<sup>+</sup> channel subunit. Biologically active portions of a mNa<sub>v</sub>1.3  $\alpha$  subunit protein can be used as targets for developing agents which modulate a Na<sup>+</sup> channel mediated activity.

In one embodiment, a biologically active portion of a mNa<sub>v</sub>1.3  $\alpha$  subunit protein comprises at least one transmembrane domain. Biologically active portions of mNa<sub>v</sub>1.3  $\alpha$  subunit proteins mediate a mNa<sub>v</sub>1.3  $\alpha$  subunit activity and include one or more features of a mNa<sub>v</sub>1.3  $\alpha$  subunit protein. Biologically active portions, in which other regions of the protein are deleted, can be prepared by recombinant techniques and evaluated for one or more of the functional activities of a native mNa<sub>v</sub>1.3  $\alpha$  subunit protein.

In one embodiment, the mNa<sub>v</sub>1.3  $\alpha$  subunit protein has an amino acid sequence shown in SEQ ID NO:2, or is substantially homologous to SEQ ID NO:2, and retains the functional activity of the protein of SEQ ID NO:2, yet differs in amino acid sequence due to natural allelic variation or mutagenesis, as described in detail in the section on nucleotides.

Accordingly, in another embodiment, the mNa<sub>v</sub>1.3  $\alpha$  subunit protein is a protein which comprises an amino acid sequence at least about 50%, 75%, 85%, 95%, 99% or more homologous to SEQ ID NO:2.

To determine the percent homology of two amino acid sequences or of two nucleic acids, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in the sequence of a first amino acid or nucleic acid sequence for optimal alignment with a second amino or nucleic acid sequence and non-homologous sequences can be disregarded for comparison purposes). The length of a reference sequence aligned for comparison purposes can be at least 50%, even 70%, 80%, or 90% of the length of the reference sequence. The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are homologous at that position (i.e., as used herein amino acid or nucleic acid "homology" is

equivalent to amino acid or nucleic acid "identity"). The percent homology between the two sequences is a function of the number of identical positions shared by the sequences (i.e., % homology= # of identical positions/total # of positions x 100).

The comparison of sequences and determination of percent homology between two sequences can be accomplished using a mathematical algorithm. A preferred, non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Karlin and Altschul *Proc. Natl. Acad. Sci. USA* 87:2264-68, 1990, modified as in Karlin and Altschul *Proc. Natl. Acad. Sci. USA* 90:5873-77, 1993. Such an algorithm is incorporated into the NBLAST and XBLAST programs (version 2.0) of Altschul, et al. *J. Mol. Biol.* 215:403-10, 1990. BLAST nucleotide searches can be performed with the NBLAST program, score=100, wordlength=12 to obtain nucleotide sequences homologous to mNa<sub>v</sub>1.3  $\alpha$  subunit nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score=50, wordlength=3 to obtain amino acid sequences homologous to mNa<sub>v</sub>1.3  $\alpha$  subunit protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al., *Nucleic Acids Res.* 25(17): 3389-3402, 1997. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used. See the website for the National Center for Biotechnology Information. Another non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller, CABIOS (1989). Such an algorithm is incorporated into the ALIGN program (version 2.0) which is part of the GCG sequence alignment software package. When utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used.

The invention also provides mNa<sub>v</sub>1.3  $\alpha$  subunit chimeric or fusion proteins. As used herein, a mNa<sub>v</sub>1.3  $\alpha$  subunit "chimeric protein" or "fusion protein" comprises a mNa<sub>v</sub>1.3  $\alpha$  subunit polypeptide operatively linked to a non-mNa<sub>v</sub>1.3  $\alpha$  subunit polypeptide. A "mNa<sub>v</sub>1.3  $\alpha$  subunit polypeptide" refers to a polypeptide having an amino acid sequence corresponding to mNa<sub>v</sub>1.3  $\alpha$  subunit, whereas a "non-mNa<sub>v</sub>1.3  $\alpha$  subunit polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a protein which is not substantially homologous to the mNa<sub>v</sub>1.3  $\alpha$  subunit protein, e.g., a protein which is different from the mNa<sub>v</sub>1.3  $\alpha$  subunit protein and which is derived from the same or a different organism, or a protein which

does not contain one or more of the features of the mNa<sub>v</sub>1.3  $\alpha$  subunit proteins described herein. Within a mNa<sub>v</sub>1.3  $\alpha$  subunit fusion protein the mNa<sub>v</sub>1.3  $\alpha$  subunit polypeptide can correspond to all or a portion of a mNa<sub>v</sub>1.3  $\alpha$  subunit protein. In a preferred embodiment, a mNa<sub>v</sub>1.3  $\alpha$  subunit fusion protein comprises at least one biologically active portion of a mNa<sub>v</sub>1.3  $\alpha$  subunit protein. In another preferred embodiment, a mNa<sub>v</sub>1.3  $\alpha$  subunit fusion protein comprises at least two biologically active portions of a mNa<sub>v</sub>1.3  $\alpha$  subunit protein. Within the fusion protein, the term "operatively linked" is intended to indicate that the mNa<sub>v</sub>1.3  $\alpha$  subunit polypeptide and the non- mNa<sub>v</sub>1.3  $\alpha$  subunit polypeptide are fused in-frame to each other. The non- mNa<sub>v</sub>1.3  $\alpha$  subunit polypeptide can be fused to the N-terminus or C-terminus of the mNa<sub>v</sub>1.3  $\alpha$  subunit polypeptide.

For example, in one embodiment, the fusion protein is a GST- mNa<sub>v</sub>1.3  $\alpha$  subunit fusion protein in which the mNa<sub>v</sub>1.3  $\alpha$  subunit sequences are fused to the C-terminus of the GST sequences. Such fusion proteins can facilitate the purification of recombinant mNa<sub>v</sub>1.3  $\alpha$  subunit.

In another embodiment, the fusion protein is a mNa<sub>v</sub>1.3  $\alpha$  subunit protein containing a heterologous signal sequence at its N-terminus. In certain host cells (e.g., mammalian host cells), expression of mNa<sub>v</sub>1.3  $\alpha$  subunit can be increased through use of a heterologous signal sequence.

The mNa<sub>v</sub>1.3  $\alpha$  subunit fusion proteins of the invention can be incorporated into pharmaceutical compositions and administered to a subject *in vivo*. The mNa<sub>v</sub>1.3  $\alpha$  subunit fusion proteins can be used to affect the bioavailability of a mNa<sub>v</sub>1.3  $\alpha$  subunit substrate. Use of mNa<sub>v</sub>1.3  $\alpha$  subunit fusion proteins may be useful therapeutically for the treatment of disorders related to Na<sup>+</sup> channel activity, e.g., neuropathic pain.

Moreover, the mNa<sub>v</sub>1.3  $\alpha$  subunit-fusion proteins of the invention can be used as immunogens to produce anti- mNa<sub>v</sub>1.3  $\alpha$  subunit antibodies in a subject, to purify mNa<sub>v</sub>1.3  $\alpha$  subunit ligands and in screening assays to identify molecules which inhibit the interaction of mNa<sub>v</sub>1.3  $\alpha$  subunit with a mNa<sub>v</sub>1.3  $\alpha$  subunit substrate.

A mNa<sub>v</sub>1.3  $\alpha$  subunit chimeric or fusion protein of the invention can be produced by standard recombinant DNA techniques. For example, DNA fragments coding for the different polypeptide sequences are ligated together in-frame in accordance with conventional techniques,

for example by employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. The fusion gene can be synthesized by conventional techniques including automated DNA synthesizers.

5 Alternatively, PCR amplification of gene fragments can be carried out using anchor primers which give rise to complementary overhangs between two consecutive gene fragments which can subsequently be annealed and reamplified to generate a chimeric gene sequence (see, for example, Current Protocols in Molecular Biology, eds. Ausubel et al. John Wiley & Sons: 1992). Moreover, many expression vectors are commercially available that already encode a fusion 10 moiety (e.g., a GST polypeptide). A mNa<sub>v</sub>1.3  $\alpha$  subunit-encoding nucleic acid can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the mNa<sub>v</sub>1.3  $\alpha$  subunit protein.

The present invention also pertains to variants of the mNa<sub>v</sub>1.3  $\alpha$  subunit proteins that function as either mNa<sub>v</sub>1.3  $\alpha$  subunit agonists (mimetics) or as mNa<sub>v</sub>1.3  $\alpha$  subunit antagonists.

15 Variants of the mNa<sub>v</sub>1.3  $\alpha$  subunit proteins can be generated by mutagenesis, e.g., discrete point mutation or truncation of a mNa<sub>v</sub>1.3  $\alpha$  subunit protein. An agonist of the mNa<sub>v</sub>1.3  $\alpha$  subunit proteins can retain substantially the same, or a subset, of the biological activities of the naturally occurring form of a mNa<sub>v</sub>1.3  $\alpha$  subunit protein. An antagonist of a mNa<sub>v</sub>1.3  $\alpha$  subunit protein can inhibit one or more of the activities of the naturally occurring form of the 20 mNa<sub>v</sub>1.3  $\alpha$  subunit protein by, for example, competitively modulating a Na<sup>+</sup> channel mediated activity of a mNa<sub>v</sub>1.3  $\alpha$  subunit protein. Thus, specific biological effects can be elicited by treatment with a variant of limited function. In one embodiment, treatment of a subject with a variant having a subset of the biological activities of the naturally occurring form of the protein has fewer side effects in a subject relative to treatment with the naturally occurring form of the 25 mNa<sub>v</sub>1.3  $\alpha$  subunit protein.

In one embodiment, variants of a mNa<sub>v</sub>1.3  $\alpha$  subunit protein which function as either mNa<sub>v</sub>1.3  $\alpha$  subunit agonists (mimetics) or as mNa<sub>v</sub>1.3  $\alpha$  subunit antagonists can be identified by screening combinatorial libraries of mutants, e.g., truncation mutants, of a mNa<sub>v</sub>1.3  $\alpha$  subunit protein for mNa<sub>v</sub>1.3  $\alpha$  subunit protein agonist or antagonist activity. In one embodiment, a 30 variegated library of mNa<sub>v</sub>1.3  $\alpha$  subunit variants is generated by combinatorial mutagenesis at

the nucleic acid level and is encoded by a variegated gene library. A variegated library of mNa<sub>v</sub>1.3  $\alpha$  subunit variants can be produced by, for example, enzymatically ligating a mixture of synthetic oligonucleotides into gene sequences such that a degenerate set of potential mNa<sub>v</sub>1.3  $\alpha$  subunit sequences is expressible as individual polypeptides, or alternatively, as a set of larger  
5 fusion proteins (e.g., for phage display) containing the set of mNa<sub>v</sub>1.3  $\alpha$  subunit sequences therein. There are a variety of methods that can be used to produce libraries of potential mNa<sub>v</sub>1.3  $\alpha$  subunit variants from a degenerate oligonucleotide sequence. Chemical synthesis of a degenerate gene sequence can be performed in an automatic DNA synthesizer, and the synthetic gene then ligated into an appropriate expression vector. Use of a degenerate set of genes allows  
10 for the provision, in one mixture, of all of the sequences encoding the desired set of potential mNa<sub>v</sub>1.3  $\alpha$  subunit sequences. Methods for synthesizing degenerate oligonucleotides are known in the art (see, e.g., Narang, S. A. *Tetrahedron* 39:3, 1983; Itakura et al. *Annu. Rev. Biochem.* 53:323, 1984; Itakura et al. *Science* 198:1056, 1984; Ike et al. *Nucleic Acid Res.* 11:477, 1983.

In addition, libraries of fragments of a mNa<sub>v</sub>1.3  $\alpha$  subunit protein coding sequence can be  
15 used to generate a variegated population of mNa<sub>v</sub>1.3  $\alpha$  subunit fragments for screening and subsequent selection of variants of a mNa<sub>v</sub>1.3  $\alpha$  subunit protein.

The mNa<sub>v</sub>1.3  $\alpha$  subunit proteins described herein can be directly or indirectly labeled with a detectable substance to facilitate detection of the bound or unbound binding agent. Suitable detectable substances include various enzymes, prosthetic groups, fluorescent materials,  
20 luminescent materials and radioactive materials.

An isolated mNa<sub>v</sub>1.3  $\alpha$  subunit protein, or a portion or fragment thereof, can be used as an immunogen to generate antibodies that bind mNa<sub>v</sub>1.3  $\alpha$  subunit using standard techniques for polyclonal and monoclonal antibody preparation. A full-length mNa<sub>v</sub>1.3  $\alpha$  subunit protein can be used or, alternatively, the invention provides antigenic peptide fragments of mNa<sub>v</sub>1.3  $\alpha$   
25 subunit for use as immunogens. The antigenic peptide of mNa<sub>v</sub>1.3  $\alpha$  subunit comprises the amino acid sequence of SEQ ID NO:2, or at least 8 amino acid residues of the amino acid sequence of SEQ ID NO:2, and encompasses an epitope of mNa<sub>v</sub>1.3  $\alpha$  subunit such that an antibody raised against the peptide forms a specific immune complex with mNa<sub>v</sub>1.3  $\alpha$  subunit. Preferably, the antigenic peptide comprises at least 10, 15, 20 or 30 amino acid residues.

A mNa<sub>v</sub>1.3  $\alpha$  subunit immunogen typically is used to prepare antibodies by immunizing a suitable subject, (e.g., rabbit, goat, mouse or other mammal) with the immunogen. An appropriate immunogenic preparation can contain, for example, recombinantly expressed mNa<sub>v</sub>1.3  $\alpha$  subunit protein or a chemically synthesized mNa<sub>v</sub>1.3  $\alpha$  subunit polypeptide. The 5 preparation can further include an adjuvant, such as Freund's complete or incomplete adjuvant, or similar immunostimulatory agent. Immunization of a suitable subject with an immunogenic mNa<sub>v</sub>1.3  $\alpha$  subunit preparation induces a polyclonal anti- mNa<sub>v</sub>1.3  $\alpha$  subunit antibody response.

Accordingly, another aspect of the invention pertains to anti-mNa<sub>v</sub>1.3  $\alpha$  subunit antibodies. The term "antibody" as used herein refers to immunoglobulin molecules and 10 immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site which specifically binds (immunoreacts with) an antigen, such as mNa<sub>v</sub>1.3  $\alpha$  subunit. Examples of immunologically active portions of immunoglobulin molecules include F(ab) and F(ab')<sub>2</sub> fragments which can be generated by treating the antibody with an enzyme such as pepsin. The invention provides polyclonal and monoclonal antibodies that bind mNa<sub>v</sub>1.3 15  $\alpha$  subunit. The term "monoclonal antibody" or "monoclonal antibody composition", as used herein, refers to a population of antibody molecules that contain only one species of an antigen binding site capable of immunoreacting with a particular epitope of mNa<sub>v</sub>1.3  $\alpha$  subunit. A monoclonal antibody composition thus typically displays a single binding affinity for a particular mNa<sub>v</sub>1.3  $\alpha$  subunit protein with which it immunoreacts.

20 Polyclonal anti- mNa<sub>v</sub>1.3  $\alpha$  subunit antibodies can be prepared as described above by immunizing a suitable subject with a mNa<sub>v</sub>1.3  $\alpha$  subunit immunogen. The anti- mNa<sub>v</sub>1.3  $\alpha$  subunit antibody titer in the immunized subject can be monitored over time by standard techniques, such as with an enzyme linked immunosorbent assay (ELISA) using immobilized mNa<sub>v</sub>1.3  $\alpha$  subunit. If desired, the antibody molecules directed against mNa<sub>v</sub>1.3  $\alpha$  subunit can 25 be isolated from the mammal (e.g., from the blood) and further purified by known techniques, such as protein A chromatography to obtain the IgG fraction. At an appropriate time after immunization, e.g., when the anti- mNa<sub>v</sub>1.3  $\alpha$  subunit antibody titers are highest, antibody-producing cells can be obtained from the subject and used to prepare monoclonal antibodies by standard techniques, such as the hybridoma technique originally described by Kohler and

30 Milstein (1975) *Nature* 256:495-497) (see also, Brown et al. *J. Immunol.* 127:539-46, 1981; Brown et al. *J. Biol. Chem.* 255:4980-83, 1980; Yeh et al. *Proc. Natl. Acad. Sci. USA* 76:2927-

31, 1976; and Yeh et al. *Int. J. Cancer* 29:269-75, 1982), the more recent human B cell hybridoma technique (Kozbor et al. *Immunol Today* 4:72, 1983), the EBV-hybridoma technique (Cole et al. *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96, 1985) or trioma techniques. The technology for producing monoclonal antibody hybridomas is well known (see generally R. H. Kenneth, in *Monoclonal Antibodies: A New Dimension In Biological Analyses*, Plenum Publishing Corp., New York, N.Y. (1980); E. A. Lerner *Yale J. Biol. Med.*, 54:387-402, 1981; M. L. Gefter et al. *Somatic Cell Genet.* 3:231-36, 1977). Briefly, an immortal cell line (typically a myeloma) is fused to lymphocytes (typically splenocytes) from a mammal immunized with a mNa<sub>v</sub>1.3  $\alpha$  subunit immunogen as described above, and the culture supernatants of the resulting hybridoma cells are screened to identify a hybridoma producing a monoclonal antibody that binds mNa<sub>v</sub>1.3  $\alpha$  subunit.

10 Any of the many well known protocols used for fusing lymphocytes and immortalized cell lines can be applied for the purpose of generating an anti- mNa<sub>v</sub>1.3  $\alpha$  subunit monoclonal antibody (see, e.g., G. Galfre et al. *Nature* 266:55052, 1977; Gefter et al. *Somatic Cell Genet.*, 15 cited supra; Lemer, *Yale J. Biol. Med.*, cited supra; Kenneth, *Monoclonal Antibodies*, cited supra). Moreover, the skilled worker will appreciate that there are many variations of such methods which also would be useful.

Alternative to preparing monoclonal antibody-secreting hybridomas, a monoclonal anti-mNa<sub>v</sub>1.3  $\alpha$  subunit antibody can be identified and isolated by screening a recombinant 20 combinatorial immunoglobulin library (e.g., an antibody phage display library) with mNa<sub>v</sub>1.3  $\alpha$  subunit to thereby isolate immunoglobulin library members that bind mNa<sub>v</sub>1.3  $\alpha$  subunit. Kits for generating and screening phage display libraries are commercially available (e.g., the Pharmacia Recombinant Phage Antibody System, Catalog No. 27-9400-01). Additionally, examples of methods and reagents particularly amenable for use in generating and screening 25 antibody display libraries can be found in, for example, Ladner et al. U.S. Pat. No. 5,223,409; Kang et al. PCT International Publication No. WO 92/18619; and McCafferty et al. *Nature* 348:552-554, 1990.

30 Additionally, recombinant anti-mNa<sub>v</sub>1.3  $\alpha$  subunit antibodies, such as chimeric and humanized monoclonal antibodies, comprising both human and non-human portions, which can be made using standard recombinant DNA techniques, are within the scope of the invention. Such chimeric and humanized monoclonal antibodies can be produced by recombinant DNA

techniques known in the art, for example using methods described in Robinson et al. International Application No. PCT/US86/02269; Akira, et al. European Patent Application 184,187; Taniguchi, M., European Patent Application 171,496; Morrison et al. European Patent Application 173,494; Neuberger et al. PCT International Publication No. WO 86/01533; Cabilly et al. U.S. Pat. No. 4,816,567; Cabilly et al. European Patent Application 125,023; Better et al. *Science* 240:1041-1043, 1988; Liu et al. *Proc. Natl. Acad. Sci. USA* 84:3439-3443, 1987; Liu et al. *J. Immunol.* 139:3521-3526, 1987; Sun et al. *Proc. Natl. Acad. Sci. USA* 84:214-218, 1987; Nishimura et al. *Canc. Res.* 47:999-1005, 1987; Wood et al. *Nature* 314:446-449, 1985; and Shaw et al. *J. Natl. Cancer Inst.* 80:1553-1559, 1988); Morrison, S. L. *Science* 229:1202-1207, 1985; Oi et al. *BioTechniques* 4:214, 1986; Winter U.S. Pat. No. 5,225,539; Jones et al. *Nature* 321:552-525, 1986; Verhoeven et al. *Science* 239:1534, 1988; and Beidler et al. *J. Immunol.* 141:4053-4060, 1988.

An anti- mNa<sub>v</sub>1.3  $\alpha$  subunit antibody (e.g., monoclonal antibody) can be used to isolate mNa<sub>v</sub>1.3  $\alpha$  subunit by standard techniques, such as affinity chromatography or immunoprecipitation. An anti-mNa<sub>v</sub>1.3  $\alpha$  subunit antibody can facilitate the purification of natural mNa<sub>v</sub>1.3  $\alpha$  subunit from cells and of recombinantly produced mNa<sub>v</sub>1.3  $\alpha$  subunit expressed in host cells. Moreover, an anti-mNa<sub>v</sub>1.3  $\alpha$  subunit antibody can be used to detect mNa<sub>v</sub>1.3  $\alpha$  subunit protein (e.g., in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the mNa<sub>v</sub>1.3  $\alpha$  subunit protein. Anti- mNa<sub>v</sub>1.3  $\alpha$  subunit antibodies can be used diagnostically to monitor protein levels in tissue as part of a clinical testing procedure, e.g., to, for example, determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling (i.e., physically linking) the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include <sup>125</sup> I, <sup>35</sup> S, or <sup>3</sup> H.

*Recombinant Expression Vectors and Host Cells*

Another aspect of the invention pertains to vectors, preferably expression vectors, containing a nucleic acid encoding a mNa<sub>v</sub>1.3  $\alpha$  subunit protein (or a portion thereof). As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other types of vectors (e.g., non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. Herein, "plasmid" and "vector" can be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The recombinant expression vectors of the invention comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, which is operatively linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operably linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner which allows for expression of the nucleotide sequence (e.g., in an in vitro transcription/translation system or in a host cell when the vector is introduced into the host cell). The term "regulatory sequence" is intended to include promoters, enhancers and other expression control elements (e.g., polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel; Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, Calif. (1990). Regulatory sequences include those which direct

constitutive expression of a nucleotide sequence in many types of host cell and those which direct expression of the nucleotide sequence only in certain host cells (e.g., tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the 5 level of expression of protein desired, and the like. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or peptides, including fusion proteins or peptides, encoded by nucleic acids as described herein (e.g., mNa<sub>v</sub>1.3  $\alpha$  subunit proteins, mutant forms of mNa<sub>v</sub>1.3  $\alpha$  subunit proteins, fusion proteins, and the like).

The recombinant expression vectors of the invention can be designed for expression of 10 mNa<sub>v</sub>1.3  $\alpha$  subunit proteins in prokaryotic or eukaryotic cells. For example, mNa<sub>v</sub>1.3  $\alpha$  subunit proteins can be expressed in bacterial cells such as *E. coli*, insect cells (using baculovirus expression vectors), yeast cells, amphibian cells, or mammalian cells. Suitable host cells are discussed further in Goeddel, *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, Calif. (1990). Alternatively, the recombinant expression vector can 15 be transcribed and translated in vitro, for example using T7 promoter regulatory sequences and T7 polymerase.

Expression of proteins in prokaryotes is most often carried out in *E. coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins.

20 Purified fusion proteins can be utilized in mNa<sub>v</sub>1.3  $\alpha$  subunit activity assays, (e.g., direct assays or competitive assays described in detail below), or to generate antibodies specific for mNa<sub>v</sub>1.3  $\alpha$  subunit, for example.

In another embodiment, the mNa<sub>v</sub>1.3  $\alpha$  subunit expression vector is a yeast expression 25 vector. Examples of vectors for expression in yeast *S. cerevisiae* include pYEpSec1 (Baldari, et al., (1987) *EMBO J.* 6:229-234), pMFA (Kurjan and Herskowitz, *Cell* 30:933-943, 1982), pJRY88 (Schultz et al., *Gene* 54:113-123, 1987), pYES2 (Invitrogen Corporation, San Diego, Calif.), and picZ (InVitrogen Corp, San Diego, Calif.).

30 Alternatively, mNa<sub>v</sub>1.3  $\alpha$  subunit proteins can be expressed in insect cells using baculovirus expression vectors. Baculovirus vectors available for expression of proteins in cultured insect cells (e.g., Sf 9 cells) include the pAc series (Smith et al. *Mol. Cell Biol.* 3:2156-2165, 1983) and the pVL series (Lucklow and Summers *Virol.* 170:31-39, 1989).

In yet another embodiment, a nucleic acid of the invention is expressed in mammalian cells using a mammalian expression vector. Examples of mammalian expression vectors include pCDM8 (Seed, B. *Nature* 329:840, 1987) and pMT2PC (Kaufman et al. *EMBO J.* 6:187-195, 1987). When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40. For other suitable expression systems for both prokaryotic and eukaryotic cells see chapters 16 and 17 of Sambrook, J., Fritsch, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual*. 2nd, ed, Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989.

In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (e.g., tissue-specific regulatory elements are used to express the nucleic acid). Tissue-specific regulatory elements are known in the art. Non-limiting examples of suitable tissue-specific promoters include the albumin promoter (liver-specific; Pinkert et al. *Genes Dev.* 1:268-277, 1987),

lymphoid-specific promoters (Calame and Eaton *Adv. Immunol.* 43:235-275, 1988), in particular promoters of T cell receptors (Winoto and Baltimore *EMBO J.* 8:729-733, 1989) and immunoglobulins (Banerji et al. *Cell* 33:729-740, 1983; Queen and Baltimore *Cell* 33:741-748, 1983), neuron-specific promoters (e.g., the neurofilament promoter; Byrne and Ruddle *Proc. Natl. Acad. Sci. USA* 86:5473-5477, 1989), pancreas-specific promoters (Edlund et al. *Science* 230:912-916, 1985), and mammary gland-specific promoters (e.g., milk whey promoter; U.S. Pat. No. 4,873,316 and European Application Publication No. 264,166). Developmentally-regulated promoters are also encompassed, for example the murine hox promoters (Kessel and Gruss *Science* 249:374-379, 1990 and the  $\alpha$ -fetoprotein promoter (Campes and Tilghman *Genes Dev.* 3:537-546, 1989).

The invention further provides a recombinant expression vector comprising a DNA molecule of the invention cloned into the expression vector in an antisense orientation. That is, the DNA molecule is operatively linked to a regulatory sequence in a manner which allows for expression (by transcription of the DNA molecule) of an RNA molecule which is antisense to mNa<sub>v</sub>1.3  $\alpha$  subunit mRNA. Regulatory sequences operatively linked to a nucleic acid cloned in the antisense orientation can be chosen which direct the continuous expression of the antisense RNA molecule in a variety of cell types, for instance viral promoters and/or enhancers, or

regulatory sequences can be chosen which direct constitutive, tissue specific or cell type specific expression of antisense RNA. The antisense expression vector can be in the form of a recombinant plasmid, phagemid or attenuated virus in which antisense nucleic acids are produced under the control of a high efficiency regulatory region, the activity of which can be determined by the cell type into which the vector is introduced. For a discussion of the regulation of gene expression using antisense genes see Weintraub, H. et al., *Antisense RNA as a molecular tool for genetic analysis, Trends in Genet.*, Vol. 1(1) 1986.

Another aspect of the invention pertains to host cells into which a nucleic acid, e.g., a mNa<sub>v</sub>1.3  $\alpha$  subunit mRNA, or a recombinant expression vector of the invention has been introduced. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

A host cell can be any prokaryotic or eukaryotic cell. For example, a mNa<sub>v</sub>1.3  $\alpha$  subunit protein can be expressed in bacterial cells such as *E. coli*, insect cells, yeast cells, *Xenopus* cells, e.g., *Xenopus* oocytes, or mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells). Other suitable host cells are known to those skilled in the art.

Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (e.g., DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation. Suitable methods for transforming or transfecting host cells can be found in Sambrook, et al. (Molecular Cloning: A Laboratory Manual. 2nd, ed, Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989), and other laboratory manuals. Nucleic acids can also be introduced by microinjection.

For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (e.g., resistance to antibiotics) is generally introduced into the host

cells along with the gene of interest. Preferred selectable markers include those which confer resistance to drugs, such as G418, hygromycin and methotrexate. Nucleic acid encoding a selectable marker can be introduced into a host cell on the same vector as that encoding a mNa<sub>v</sub>1.3  $\alpha$  subunit protein or can be introduced on a separate vector. Cells stably transfected with the introduced nucleic acid can be identified by drug selection (e.g., cells that have incorporated the selectable marker gene will survive, while the other cells die).

5 A host cell of the invention, such as a prokaryotic or eukaryotic host cell in culture, can be used to produce (i.e., express) a mNa<sub>v</sub>1.3  $\alpha$  subunit protein. Accordingly, the invention further provides methods for producing a mNa<sub>v</sub>1.3  $\alpha$  subunit protein using the host cells of the invention.

10 The nucleic acid molecules of the invention can be inserted into vectors and used as gene therapy vectors. Gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (see U.S. Pat. No. 5,328,470) or by stereotactic injection (see e.g., Chen et al. *Proc. Natl. Acad. Sci. USA* 91:3054-3057, 1994). The pharmaceutical preparation of 15 the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, e.g., retroviral vectors, the pharmaceutical preparation can include one or more cells which produce the gene delivery system.

#### 20 *Uses and Methods of the Invention*

The nucleic acid molecules, proteins, protein homologues, and antibodies described herein can be used in one or more of the following methods: a) screening assays; b) predictive medicine (e.g., diagnostic assays, prognostic assays, monitoring clinical trials, and 25 pharmacogenetics); and c) methods of treatment (e.g., therapeutic and prophylactic).

The isolated nucleic acid molecules of the invention can be used, for example, to express mNa<sub>v</sub>1.3  $\alpha$  subunit protein (e.g., via a recombinant expression vector in a host cell in gene therapy applications), to detect mNa<sub>v</sub>1.3  $\alpha$  subunit mRNA (e.g., in a biological sample) or a 30 genetic alteration in a gene encoding a mNa<sub>v</sub>1.3  $\alpha$  subunit protein, and to modulate mNa<sub>v</sub>1.3  $\alpha$  subunit activity, as described further below. The mNa<sub>v</sub>1.3  $\alpha$  subunit proteins can be used to treat disorders characterized by insufficient or excessive production of a mNa<sub>v</sub>1.3  $\alpha$  subunit substrate

or production of mNa<sub>v</sub>1.3  $\alpha$  subunit inhibitors. In addition, the mNa<sub>v</sub>1.3  $\alpha$  subunit proteins can be used to screen for naturally occurring mNa<sub>v</sub>1.3  $\alpha$  subunit substrates, to screen for drugs or compounds which modulate mNa<sub>v</sub>1.3  $\alpha$  subunit activity, as well as to treat disorders characterized by insufficient or excessive production of mNa<sub>v</sub>1.3  $\alpha$  subunit protein or production 5 of mNa<sub>v</sub>1.3  $\alpha$  subunit protein forms which have decreased or aberrant activity compared to mNa<sub>v</sub>1.3  $\alpha$  subunit wild type protein. Moreover, the anti-mNa<sub>v</sub>1.3  $\alpha$  subunit antibodies of the invention can be used to detect and isolate mNa<sub>v</sub>1.3  $\alpha$  subunit proteins and modulate mNa<sub>v</sub>1.3  $\alpha$  subunit activity.

#### *Screening Assays*

10 The invention provides methods for identifying modulators, i.e., candidate or test compounds or agents (e.g., proteins, peptides, peptidomimetics, peptoids, small molecules or other drugs) which bind to Na<sup>+</sup> channels comprising a mNa<sub>v</sub>1.3  $\alpha$  subunit described herein. Compounds thus identified can be used to modulate the activity of these Na<sup>+</sup> channels e.g., in a therapeutic protocol.

15 In one embodiment, the invention provides assays for screening test compounds which are substrates of Na<sup>+</sup> channels that include a mNa<sub>v</sub>1.3  $\alpha$  subunit described herein, or a biologically active portion of the subunit. In another embodiment, the invention provides assays for screening candidate or test compounds that bind to or modulate an activity of these Na<sup>+</sup> channels.

20 Ion channel-modulating compounds can be identified through both *in vitro* (e.g., cell and non-cell based) and *in vivo* methods. In one embodiment, ion influx assays are used to measure Na<sup>+</sup> channel activity.

Assays to measure ion channel activity include flux assays, patch-clamp 25 electrophysiology, and two electrode voltage clamp electrophysiology (see, e.g., Lin et al., *Neuron* 18:153-166, 1997). Patch-clamp physiology can be performed as follows. Briefly, a pipette tip containing a small electrode is pressed against a cell membrane to create a tight seal between the pipette and the membrane. The electrode captures the ions flowing through the membrane defined by the edges of the pipette tip. Various configurations can be employed to measure currents within the cell or within a patch of membrane or over the entire cell.

Two electrode voltage-clamp (TEVC) physiology can be performed as follows. Briefly, two sharp microelectrodes are pressed through a cell membrane. One electrode monitors membrane potential and the other electrode injects current to hold the membrane potential at the desired level. Both patch-clamp and TEVC techniques provide information regarding both 5 kinetics and intensity of ion channel currents.

High-throughput electrophysiology can be performed, e.g., as described in U.S. 6,268,168 and U.S. 6,048,722.

Assays that measure changes in ion concentration can be used. For example, the test system can be loaded with detectable  $\text{Na}^+$  (e.g., radiolabeled  $\text{Na}^+$ ). Detection of the  $\text{Na}^+$  can give 10 an indication of a change in  $\text{Na}^+$  concentration. In one embodiment, the assay involves detection of  $\text{Na}^+$  following stimulation by application of a voltage to the test system (e.g., a cell or an enclosed membrane preparation). Sodium-sensitive dyes, such as sodium green or corona red, may also be used to measure changes in ion concentration.

In one embodiment,  $\text{Na}^+$  channel modulation is assayed using a *Xenopus* oocyte system. 15 For a detailed description of transient expression of ion channels and recording from *Xenopus* oocytes, see, e.g., Xu and Lipscombe, *J. Neurosci.* 21(16):5944-5951, 2001; Lin et al., *supra*. In another embodiment, the assay is a mammalian-cell based assay, e.g., using a human or mouse cell. A particular  $\text{Na}^+$  channel, such as a  $\text{mNa}_v1.3 \alpha$  subunit channel, can be studied in isolation by transfection into a cell type that does not express other  $\text{Na}^+$  channels.

## 20 Compounds

The test compounds of the present invention can be obtained singly or using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; peptoid libraries (libraries of molecules having the functionalities of peptides, but with a novel, non-peptide backbone which are resistant to enzymatic degradation but which 25 nevertheless remain bioactive; see, e.g., Zuckermann, R.N. *et al.*, *J. Med. Chem.* 37:2678-85, 1994); spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the 'one-bead one-compound' library method; and synthetic library methods using affinity chromatography selection. The biological library and peptoid library approaches are limited to peptide libraries, while the other four approaches are applicable 30 to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam, *Anticancer Drug Des.* 12:145, 1997).

Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 90:6909, 1993; Erb *et al.*, *Proc. Natl. Acad. Sci. USA* 91:11422, 1994; Zuckermann *et al.*, *J. Med. Chem.* 37:2678, 1994; Cho *et al.*, *Science* 261:1303, 1993; Carrell *et al.*, *Angew. Chem. Int. Ed. Engl.* 33:2059, 1994; Carell *et al.*, *Angew. Chem. Int. Ed. Engl.* 33:2061, 1994; and Gallop *et al.*, *J. Med. Chem.* 37:1233, 1994.

Libraries of compounds may be presented in solution (e.g., Houghten, *Biotechniques* 13:412-421, 1992), or on beads (Lam, *Nature* 354:82-84, 1991), chips (Fodor, *Nature* 364:555-556, 1993), bacteria (Ladner, U.S. Patent No. 5,223,409), spores (Ladner U.S. Patent No. 5,223,409), plasmids (Cull *et al.*, *Proc Natl Acad Sci USA* 89:1865-1869, 1992) or on phage (Scott and Smith, *Science* 249:386-390, 1990; Devlin, *Science* 249:404-406, 1990; Cwirla *et al.* *Proc. Natl. Acad. Sci.* 87:6378-6382, 1990; Felici, *J. Mol. Biol.* 222:301-310, 1991; Ladner *supra*).

Chemical compounds to be used as test compounds (i.e., potential inhibitor, antagonist, agonist) can be obtained from commercial sources or can be synthesized from readily available starting materials using standard synthetic techniques and methodologies known to those of ordinary skill in the art. Synthetic chemistry transformations and protecting group methodologies (protection and deprotection) useful in synthesizing the compounds identified by the methods described herein are known in the art and include, for example, those such as described in R. Larock, *Comprehensive Organic Transformations*, VCH Publishers (1989); T. W. Greene and P. G. M. Wuts, *Protective Groups in Organic Synthesis*, 2nd ed., John Wiley and Sons (1991); L. Fieser and M. Fieser, *Fieser and Fieser's Reagents for Organic Synthesis*, John Wiley and Sons (1994); and L. Paquette, ed., *Encyclopedia of Reagents for Organic Synthesis*, John Wiley and Sons (1995), and subsequent editions thereof.

In one aspect the compounds are organic small molecules, that is, compounds having molecular weight less than 1,000 amu, alternatively between 350-750 amu. In other aspects, the compounds are: (i) those that are non-peptidic; (ii) those having between 1 and 5, inclusive, heterocyclyl, or heteroaryl ring groups, which may bear further substituents; (iii) those in their respective pharmaceutically acceptable salt forms; or (iv) those that are peptidic.

The term "heterocyclyl" refers to a nonaromatic 3-8 membered monocyclic, 8-12 membered bicyclic, or 11-14 membered tricyclic ring system having 1-3 heteroatoms if monocyclic, 1-6 heteroatoms if bicyclic, or 1-9 heteroatoms if tricyclic, said heteroatoms

selected from O, N, or S (e.g., carbon atoms and 1-3, 1-6, or 1-9 heteroatoms of N, O, or S if monocyclic, bicyclic, or tricyclic, respectively), wherein 0, 1, 2 or 3 atoms of each ring can be substituted by a substituent.

The term "heteroaryl" refers to an aromatic 5-8 membered monocyclic, 8-12 membered 5 bicyclic, or 11-14 membered tricyclic ring system having 1-3 heteroatoms if monocyclic, 1-6 heteroatoms if bicyclic, or 1-9 heteroatoms if tricyclic, said heteroatoms selected from O, N, or S (e.g., carbon atoms and 1-3, 1-6, or 1-9 heteroatoms of N, O, or S if monocyclic, bicyclic, or tricyclic, respectively), wherein 0, 1, 2, 3, or 4 atoms of each ring can be substituted by a substituent.

10 The term "substituents" refers to a group "substituted" on an alkyl, cycloalkyl, aryl, heterocycl, or heteroaryl group at any atom of that group. Suitable substituents include, without limitation, alkyl, alkenyl, alkynyl, alkoxy, halo, hydroxy, cyano, nitro, amino,  $\text{SO}_3\text{H}$ , perfluoroalkyl, perfluoroalkoxy, methylenedioxy, ethylenedioxy, carboxyl, o xo, thioxo, imino (alkyl, aryl, aralkyl),  $\text{S}(\text{O})_n$ alkyl (where n is 0-2),  $\text{S}(\text{O})_n$  aryl (where n is 0-2),  $\text{S}(\text{O})_n$  heteroaryl 15 (where n is 0-2),  $\text{S}(\text{O})_n$  heterocycl (where n is 0-2), amine (mono-, di-, alkyl, cycloalkyl, aralkyl, heteroaralkyl, and combinations thereof), ester (alkyl, aralkyl, heteroaralkyl), amide (mono-, di-, alkyl, aralkyl, heteroaralkyl, and combinations thereof), sulfonamide (mono-, di-, alkyl, aralkyl, heteroaralkyl, and combinations thereof), unsubstituted aryl, unsubstituted heteroaryl, unsubstituted heterocycl, and unsubstituted cycloalkyl. In one aspect, the 20 substituents on a group are independently any one single, or any subset of the aforementioned substituents.

25 Combinations of substituents and variables in compounds envisioned by this invention are only those that result in the formation of stable compounds. The term "stable", as used herein, refers to compounds which possess stability sufficient to allow manufacture and which maintains the integrity of the compound for a sufficient period of time to be useful for the purposes detailed herein (e.g., transport, storage, assaying, therapeutic administration to a subject).

30 Pharmaceutically acceptable salts of the compounds herein include those derived from pharmaceutically acceptable inorganic and organic acids and bases. Examples of suitable acid salts include acetate, adipate, alginate, aspartate, benzoate, benzenesulfonate, bisulfate, butyrate, citrate, digluconate, ethanesulfonate, formate, fumarate, glycolate, hemisulfate, heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, lactate, maleate, malonate,

methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, palmoate, pectinate, persulfatephosphate, picrate, pivalate, propionate, salicylate, succinate, sulfate, tartrate, thiocyanate, tosylate and undecanoate.

The compounds described herein can contain one or more asymmetric centers and thus  
5 occur as racemates and racemic mixtures, single enantiomers, individual diastereomers and diastereomeric mixtures. All such isomeric forms of these compounds are expressly included in the present invention. The compounds described herein can also be represented in multiple tautomeric forms, all of which are included herein. The compounds can also occur in cis-or trans-or E-or Z-double bond isomeric forms. All such isomeric forms of such compounds are  
10 expressly included in the present invention.

#### Binding Assays

The ability of the test compound to bind to a  $\text{Na}^+$  channel comprising a  $\text{mNa}_v1.3 \alpha$  subunit can also be evaluated. While  $\text{Na}^+$  channel binding is not a prerequisite for channel modulatory activity, compounds that bind a  $\text{Na}^+$  channel can be useful in modulating activity of the channel. This can be accomplished, for example, by coupling the compound, e.g., the substrate, with a radioisotope or enzymatic label such that binding of the compound, e.g., the substrate, to  $\text{Na}^+$  channels can be determined by detecting the labeled compound, e.g., substrate, in a complex. Alternatively, a  $\text{Na}^+$  channel comprising a  $\text{mNa}_v1.3 \alpha$  subunit described herein could be coupled with a radioisotope or enzymatic label to monitor the ability of a test compound to modulate the complex. For example, compounds can be labeled with  $^{125}\text{I}$ ,  $^{35}\text{S}$ ,  $^{14}\text{C}$ , or  $^3\text{H}$ , either directly or indirectly, and the radioisotope detected by direct counting of radioemission or by scintillation counting. Alternatively, compounds can be enzymatically labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product.

25 The ability of a test compound to interact with a  $\text{Na}^+$  channel comprising a  $\text{mNa}_v1.3 \alpha$  subunit or without the labeling of any of the interactants can be evaluated. For example, a microphysiometer can be used to detect the interaction of a compound with a  $\text{Na}^+$  channel without the labeling of either the compound or the  $\text{Na}^+$  channel (McConnell, H. M. *et al.*, *Science* 257:1906-1912, 1992). As used herein, a "microphysiometer" (e.g., Cytosensor) is an  
30 analytical instrument that measures the rate at which a cell acidifies its environment using a

light-addressable potentiometric sensor (LAPS). Changes in this acidification rate can be used as an indicator of the interaction between a compound and a  $\text{Na}^+$  channel.

In yet another embodiment, a cell-free assay is provided in which a  $\text{Na}^+$  channel described herein or biologically active portion thereof is contacted with a test compound and the ability of the test compound to bind to the channel or biologically active portion thereof is evaluated. Preferably, the cell-free assay comprises a membrane. Cell-free assays involve preparing a reaction mixture of the target gene protein and the test compound under conditions and for a time sufficient to allow the two components to interact and bind, thus forming a complex that can be removed and/or detected.

The interaction between two molecules can also be detected, e.g., using fluorescence energy transfer (FET) (see, for example, Lakowicz *et al.*, U.S. Patent No. 5,631,169; Stavrianopoulos *et al.*, U.S. Patent No. 4,868,103). A fluorophore label on the first, 'donor' molecule is selected such that its emitted fluorescent energy will be absorbed by a fluorescent label on a second, 'acceptor' molecule, which in turn is able to fluoresce due to the absorbed energy. Alternately, the 'donor' protein molecule may simply utilize the natural fluorescent energy of tryptophan residues. Labels are chosen that emit different wavelengths of light, such that the 'acceptor' molecule label may be differentiated from that of the 'donor'. Since the efficiency of energy transfer between the labels is related to the distance separating the molecules, the spatial relationship between the molecules can be assessed. In a situation in which binding occurs between the molecules, the fluorescent emission of the 'acceptor' molecule label in the assay should be maximal. An FET binding event can be conveniently measured through standard fluorometric detection means well known in the art (e.g., using a fluorimeter).

In another embodiment, determining the ability of a test compound to bind to a  $\text{Na}^+$  channel described herein can be accomplished using real-time Biomolecular Interaction Analysis (BIA) (see, e.g., Sjolander, S. and Urbaniczky, C., *Anal. Chem.* 63:2338-2345, 1991; and Szabo *et al.*, *Curr. Opin. Struct. Biol.* 5:699-705, 1995). "Surface plasmon resonance" or "BIA" detects biospecific interactions in real time, without labeling any of the interactants (e.g., BIACore). Changes in the mass at the binding surface (indicative of a binding event) result in alterations of the refractive index of light near the surface (the optical phenomenon of surface plasmon resonance (SPR)), resulting in a detectable signal that can be used as an indication of real-time reactions between biological molecules.

In one embodiment, the sample comprising the  $\text{Na}^+$  channel or the test compound is anchored onto a solid phase. The channel/test compound complexes anchored on the solid phase can be detected at the end of the reaction.

It may be desirable to immobilize either the  $\text{Na}^+$  channel, an anti- $\text{Na}^+$  channel antibody or its target molecule to facilitate separation of complexed from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay. Binding of a test compound to a  $\text{Na}^+$  channel, or interaction of a  $\text{Na}^+$  channel with a target molecule in the presence and absence of a candidate compound, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtiter plates, test tubes, and micro-centrifuge tubes. In one embodiment, a fusion protein can be provided which adds a domain that allows one or both of the proteins to be bound to a matrix. For example, glutathione-S-transferase/m $\text{Na}_v$ 1.3  $\alpha$  subunit fusion proteins or glutathione-S-transferase/target fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, MO) or glutathione derivatized microtiter plates, which are then combined with the test compound or the test compound and a sample comprising the  $\text{Na}^+$  channel comprising the GST-tagged subunit, and the mixture incubated under conditions conducive to complex formation (e.g., at physiological conditions for salt and pH). Following incubation, the beads or microtiter plate wells are washed to remove any unbound components, the matrix immobilized in the case of beads, complex determined either directly or indirectly, for example, as described above.

Other techniques for immobilizing a complex of  $\text{Na}^+$  channel subunits on matrices include using conjugation of biotin and streptavidin. For example, biotinylated m $\text{Na}_v$ 1.3  $\alpha$  subunit proteins can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques known in the art (e.g., biotinylation kit, Pierce Chemicals, Rockford, IL), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical).

In order to conduct the assay, the non-immobilized component is added to the coated surface containing the anchored component. After the reaction is complete, unreacted components are removed (e.g., by washing) under conditions such that any complexes formed will remain immobilized on the solid surface. The detection of complexes anchored on the solid surface can be accomplished in a number of ways. Where the previously non-immobilized component is pre-labeled, the detection of label immobilized on the surface indicates that complexes were formed. Where the previously non-immobilized component is not pre-labeled,

an indirect label can be used to detect complexes anchored on the surface; e.g., using a labeled antibody specific for the immobilized component (the antibody, in turn, can be directly labeled or indirectly labeled with, e.g., a labeled anti-Ig antibody).

5 In one embodiment, this assay is performed utilizing antibodies reactive with an epitope on the  $\text{Na}^+$  channel but which do not interfere with binding of the channel to a target molecule. Such antibodies can be derivatized to the wells of the plate, and unbound target or  $\text{Na}^+$  channels trapped in the wells by antibody conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies reactive with a component of the  $\text{Na}^+$  channel, as well as enzyme-linked assays which rely on detecting an enzymatic activity associated with the channel.

10 Alternatively, cell free assays can be conducted in a liquid phase. In such an assay, the reaction products are separated from unreacted components, by any of a number of standard techniques, including but not limited to: differential centrifugation (see, for example, Rivas, G., and Minton, A.P., *Trends Biochem Sci* 18:284-7, 1993); chromatography (gel filtration chromatography, ion-exchange chromatography); electrophoresis and immunoprecipitation (see, for example, Ausubel, F. *et al.*, eds. (1999) *Current Protocols in Molecular Biology*, J. Wiley: New York). Such resins and chromatographic techniques are known to one skilled in the art (see, e.g., Heegaard, N.H., *J Mol Recognit* 11:141-8, 1998; Hage, D.S., and Tweed, S.A., *J Chromatogr B Biomed Sci Appl.* 699:499-525, 1997). Further, fluorescence energy transfer may also be conveniently utilized, as described herein, to detect binding without further purification of the complex from solution. Preferably, cell free assays preserve the structure of the  $\text{Na}^+$  channel complex, e.g., by including a membrane component or synthetic membrane components.

15 In a preferred embodiment, the assay includes contacting the  $\text{Na}^+$  channel or channel comprising biologically active portions of the m $\text{Na}_v1.3$   $\alpha$  subunit with a known compound which binds the channel to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with a  $\text{Na}^+$  channel, wherein determining the ability of the test compound to interact with a  $\text{Na}^+$  channel includes determining the ability of the test compound to preferentially bind to the  $\text{Na}^+$  channel, or to modulate the activity of the channel, as compared to the known compound.

20 30 The  $\text{Na}^+$  channels described herein can, *in vivo*, interact with one or more cellular or extracellular macromolecules, such as proteins. For the purposes of this discussion, such cellular

and extracellular macromolecules are referred to herein as "binding partners." Compounds that disrupt such interactions can be useful in regulating the activity of the target gene product. Such compounds can include, but are not limited to molecules such as antibodies, peptides, and small molecules.

5 To identify compounds that interfere with the interaction between the  $\text{Na}^+$  channel and an extracellular binding partner(s), a reaction mixture containing the target gene product and the binding partner is prepared, under conditions and for a time sufficient, to allow the two products to form a complex. In order to test an inhibitory agent, the reaction mixture is provided in the presence and absence of the test compound. The test compound can be initially included in the  
10 reaction mixture, or can be added at a time subsequent to the addition of the  $\text{Na}^+$  channel and its cellular or extracellular binding partner. Control reaction mixtures are incubated without the test compound or with a placebo. The formation of any complexes between the  $\text{Na}^+$  channel and the cellular or extracellular binding partner is then detected. The formation of a complex in the control reaction, but not in the reaction mixture containing the test compound, indicates that the  
15 compound interferes with the interaction of the target gene product and the interactive binding partner. Additionally, complex formation within reaction mixtures containing the test compound and normal target gene product can also be compared to complex formation within reaction mixtures containing the test compound and a  $\text{Na}^+$  channel comprising one or more mutant subunits. This comparison can be important in those cases wherein it is desirable to identify  
20 compounds that disrupt interactions of mutant but not normal target gene products.

These assays can be conducted in a heterogeneous or homogeneous format.

Heterogeneous assays involve anchoring either the  $\text{Na}^+$  channel or the binding partner onto a solid phase, and detecting complexes anchored on the solid phase at the end of the reaction. In homogeneous assays, the entire reaction is carried out in a liquid phase. In either approach, the  
25 order of addition of reactants can be varied to obtain different information about the compounds being tested. For example, test compounds that interfere with the interaction between the target gene products and the binding partners, e.g., by competition, can be identified by conducting the reaction in the presence of the test substance. Alternatively, test compounds that disrupt preformed complexes, e.g., compounds with higher binding constants that displace one of the  
30 components from the complex, can be tested by adding the test compound to the reaction mixture after complexes have been formed. The various formats are briefly described below.

In a heterogeneous assay system, either the target gene product or the interactive cellular or extracellular binding partner is anchored onto a solid surface (e.g., a microtiter plate), while the non-anchored species is labeled, either directly or indirectly. The anchored species can be immobilized by non-covalent or covalent attachments. Alternatively, an immobilized antibody specific for the species to be anchored can be used to anchor the species to the solid surface.

In order to conduct the assay, the partner of the immobilized species is exposed to the coated surface with or without the test compound. After the reaction is complete, unreacted components are removed (e.g., by washing) and any complexes formed will remain immobilized on the solid surface. Where the non-immobilized species is pre-labeled, the detection of label immobilized on the surface indicates that complexes were formed. Where the non-immobilized species is not pre-labeled, an indirect label can be used to detect complexes anchored on the surface; e.g., using a labeled antibody specific for the initially non-immobilized species (the antibody, in turn, can be directly labeled or indirectly labeled with, e.g., a labeled anti-Ig antibody). Depending upon the order of addition of reaction components, test compounds that inhibit complex formation or that disrupt preformed complexes can be detected.

Alternatively, the reaction can be conducted in a liquid phase in the presence or absence of the test compound, the reaction products separated from unreacted components, and complexes detected; e.g., using an immobilized antibody specific for one of the binding components to anchor any complexes formed in solution, and a labeled antibody specific for the other partner to detect anchored complexes. Again, depending upon the order of addition of reactants to the liquid phase, test compounds that inhibit complex or that disrupt preformed complexes can be identified.

In an alternate embodiment of the invention, a homogeneous assay can be used. For example, a preformed complex of the target gene product and the interactive cellular or extracellular binding partner product is prepared in that either the  $\text{Na}^+$  channel subunits or their binding partners are labeled, but the signal generated by the label is quenched due to complex formation (see, e.g., U.S. Patent No. 4,109,496 that utilizes this approach for immunoassays). The addition of a test substance that competes with and displaces one of the species from the preformed complex will result in the generation of a signal above background. In this way, test substances that disrupt target gene product-binding partner interaction can be identified.

In yet another aspect, the  $\text{Na}^+$  channel proteins or fragments thereof can be used as "bait proteins" in a two-hybrid assay or three-hybrid assay (see, e.g., U.S. Patent No. 5,283,317; Zervos *et al.*, *Cell* 72:223-232, 1993; Madura *et al.*, *J. Biol. Chem.* 268:12046-12054, 1993; Bartel *et al.*, *Biotechniques* 14:920-924, 1993; Iwabuchi *et al.*, *Oncogene* 8:1693-1696, 1993; and Brent WO94/10300), to identify other proteins, which bind to or interact with  $\text{Na}^+$  channel proteins ("Na<sup>+</sup> channel-binding proteins" or "Na<sup>+</sup> channel-bps") and are involved in  $\text{Na}^+$  channel activity. Such  $\text{Na}^+$  channel-bps can be activators or inhibitors of signals by the  $\text{Na}^+$  channels or  $\text{Na}^+$ -sensitive targets.

The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. In one construct, the gene that codes for a  $\text{Na}^+$  channel subunitprotein or fragment thereof (e.g., corresponding to a soluble portion of an extracellular domain of the subunit) is fused to a gene encoding the DNA binding domain of a known transcription factor (e.g., GAL-4). In the other construct, a DNA sequence, from a library of DNA sequences, which encodes an unidentified protein ("prey" or "sample") is fused to a gene that codes for the activation domain of the known transcription factor. (Alternatively, the  $\text{Na}^+$  channel subunit can be the fused to the activator domain.) If the "bait" and the "prey" proteins are able to interact, *in vivo*, forming a  $\text{Na}^+$  channel subunit-dependent complex, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (e.g., lacZ) that is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected and cell colonies containing the functional transcription factor can be isolated and used to obtain the cloned gene that encodes the protein that interacts with the  $\text{Na}^+$  channel subunit.

In another embodiment, modulators of  $\text{Na}^+$  channel subunit expression are identified. For example, a cell or cell free mixture is contacted with a candidate compound and the expression of m $\text{Na}_v$ 1.3  $\alpha$  subunit mRNA or protein evaluated relative to the level of expression of m $\text{Na}_v$ 1.3  $\alpha$  subunit mRNA or protein in the absence of the candidate compound. When expression of m $\text{Na}_v$ 1.3  $\alpha$  subunit mRNA or protein is greater in the presence of the candidate compound than in its absence, the candidate compound is identified as a stimulator of m $\text{Na}_v$ 1.3  $\alpha$  subunit mRNA or protein expression. Alternatively, when expression of m $\text{Na}_v$ 1.3  $\alpha$  subunit mRNA or protein is

less (statistically significantly less) in the presence of the candidate compound than in its absence, the candidate compound is identified as an inhibitor of mNa<sub>v</sub>1.3  $\alpha$  subunit mRNA or protein expression. The level of mNa<sub>v</sub>1.3  $\alpha$  subunit mRNA or protein expression can be determined by methods described herein for detecting mNa<sub>v</sub>1.3  $\alpha$  subunit mRNA or protein.

5 In another aspect, the invention pertains to a combination of two or more of the assays described herein. For example, a modulating agent can be identified using a cell-based or a cell free assay, and the ability of the agent to modulate the activity of a Na<sup>+</sup> channel can be confirmed *in vivo*, e.g., in an animal such as an animal model for a pain disorder or a disorder associated with stroke or traumatic brain injury.

10 This invention further pertains to novel agents identified by the above-described screening assays. Accordingly, it is within the scope of this invention to further use an agent identified as described herein (e.g., a mNa<sub>v</sub>1.3  $\alpha$  subunit channel-modulating agent, an antisense nucleic acid molecule corresponding to one or more of the Na<sup>+</sup> channel subunits described herein, a channel-specific antibody, or a mNa<sub>v</sub>1.3  $\alpha$  subunit channel-binding partner) in an 15 appropriate animal model to determine the efficacy, toxicity, side effects, or mechanism of action, of treatment with such an agent. Furthermore, novel agents identified by the above-described screening assays can be used for treatments as described herein.

Diagnostic and prognostic assays of the invention include methods for assessing the expression level of mNa<sub>v</sub>1.3  $\alpha$  subunit and for identifying variations and mutations in the 20 nucleotide or amino acid sequence of mNa<sub>v</sub>1.3  $\alpha$  subunit molecules.

Expression Monitoring and Profiling. The presence, level, or absence of mNa<sub>v</sub>1.3  $\alpha$  subunit protein or nucleic acid in a biological sample can be evaluated by obtaining a biological sample from a test subject and contacting the biological sample with a compound or an agent capable of detecting mNa<sub>v</sub>1.3  $\alpha$  subunit protein or nucleic acid (e.g., mRNA, genomic DNA) that 25 encodes mNa<sub>v</sub>1.3  $\alpha$  subunit protein such that the presence of the protein or nucleic acid is detected in the biological sample. The term "biological sample" includes tissues, cells and biological fluids isolated from a subject, as well as tissues, cells and fluids present within a subject. A preferred biological sample is brain tissue. The level of expression of the mNa<sub>v</sub>1.3  $\alpha$  subunit gene can be measured in a number of ways, including, but not limited to: measuring the 30 mRNA encoded by the mNa<sub>v</sub>1.3  $\alpha$  subunit gene; measuring the amount of protein encoded by

the mNa<sub>v</sub>1.3  $\alpha$  subunit gene; or measuring the activity of the protein encoded by the mNa<sub>v</sub>1.3  $\alpha$  subunit.

The level of mRNA corresponding to a mNa<sub>v</sub>1.3  $\alpha$  subunit gene in a cell can be determined both by *in situ* and by *in vitro* formats.

5 The isolated mRNA can be used in hybridization or amplification assays that include, but are not limited to, Southern or Northern analyses, polymerase chain reaction analyses and probe arrays. One preferred diagnostic method for the detection of mRNA levels involves contacting the isolated mRNA with a nucleic acid molecule (probe) that can hybridize to the mRNA encoded by the gene being detected. The nucleic acid probe can be, for example, a full-length 10 Na<sup>+</sup> channel a subunitnucleic acid, such as the nucleic acids described herein, or a portion thereof, such as an oligonucleotide of at least 7, 15, 30, 50, 100, 250 or 500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to Na<sup>+</sup> channel a subunitmRNA or genomic DNA. The probe can be disposed on an address of an array, e.g., an array described below. Other suitable probes for use in the diagnostic assays are described 15 herein.

In one format, mRNA (or cDNA) is immobilized on a surface and contacted with the probes, for example by running the isolated mRNA on an agarose gel and transferring the mRNA from the gel to a membrane, such as nitrocellulose. In an alternative format, the probes are immobilized on a surface and the mRNA (or cDNA) is contacted with the probes, for example, 20 in a two-dimensional gene chip array described below. A skilled artisan can adapt known mRNA detection methods for use in detecting the level of mRNA encoded by the mNa<sub>v</sub>1.3  $\alpha$  subunit genes.

The level of mRNA in a sample that is encoded by a mNa<sub>v</sub>1.3  $\alpha$  subunit gene can be evaluated with nucleic acid amplification, e.g., by rtPCR (Mullis (1987) U.S. Patent No. 25 4,683,202), ligase chain reaction (Barany, *Proc. Natl. Acad. Sci. USA* 88:189-193, 1991), self - sustained sequence replication (Guatelli *et al.*, *Proc. Natl. Acad. Sci. USA* 87:1874-1878, 1990), transcriptional amplification system (Kwoh *et al.*, *Proc. Natl. Acad. Sci. USA* 86:1173-1177, 1989), Q-Beta Replicase (Lizardi *et al.*, *Bio/Technology* 6:1197, 1988), rolling circle replication (Lizardi *et al.*, U.S. Patent No. 5,854,033) or any other nucleic acid amplification method, 30 followed by the detection of the amplified molecules using techniques known in the art. As used herein, amplification primers are defined as being a pair of nucleic acid molecules that can

anneal to 5' or 3' regions of a gene (plus and minus strands, respectively, or vice-versa) and contain a short region in between. In general, amplification primers are from about 10 to 30 nucleotides in length and flank a region from about 50 to 200 nucleotides in length. Under appropriate conditions and with appropriate reagents, such primers permit the amplification of a nucleic acid molecule comprising the nucleotide sequence flanked by the primers.

5 For *in situ* methods, a cell or tissue sample can be prepared/processed and immobilized on a support, typically a glass slide, and then contacted with a probe that can hybridize to mRNA that encodes the mNa<sub>v</sub>1.3  $\alpha$  subunit gene being analyzed.

10 In another embodiment, the methods herein include further contacting a control sample with a compound or agent capable of detecting mNa<sub>v</sub>1.3  $\alpha$  subunit mRNA, or genomic DNA, and comparing the presence of mNa<sub>v</sub>1.3  $\alpha$  subunit mRNA or genomic DNA in the control sample with the presence of mNa<sub>v</sub>1.3  $\alpha$  subunit mRNA or genomic DNA in the test sample. In still another embodiment, serial analysis of gene expression, as described in U.S. Patent No. 5,695,937, is used to detect mNa<sub>v</sub>1.3  $\alpha$  subunit transcript levels.

15 A variety of methods can be used to determine the level of protein encoded by mNa<sub>v</sub>1.3  $\alpha$  subunit genes. In general, these methods include contacting an agent that selectively binds to the protein, such as an antibody with a sample, to evaluate the level of protein in the sample. In a preferred embodiment, the antibody bears a detectable label. Antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (e.g., Fab or F(ab')<sub>2</sub>) can be used. The term "labeled", with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (i.e., physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with a detectable substance. Examples of detectable substances are provided herein.

20 The detection methods can be used to detect mNa<sub>v</sub>1.3  $\alpha$  subunit protein in a biological sample *in vitro* as well as *in vivo*. *In vitro* techniques for detection of proteins include enzyme linked immunosorbent assays (ELISAs), immunoprecipitations, immunofluorescence, enzyme immunoassay (EIA), radioimmunoassay (RIA), and Western blot analysis. *In vivo* techniques for detection of proteins include introducing into a subject a labeled anti-mNa<sub>v</sub>1.3  $\alpha$  subunit antibody. For example, the antibody can be labeled with a radioactive marker whose presence 30 and location in a subject can be detected by standard imaging techniques. In another embodiment, the sample is labeled, e.g., biotinylated and then contacted to the antibody, e.g., an

anti-mNa<sub>v</sub>1.3  $\alpha$  subunit antibody positioned on an antibody array (as described below). The sample can be detected, e.g., with avidin coupled to a fluorescent label.

In another embodiment, the methods further include contacting the control sample with a compound or agent capable of detecting a mNa<sub>v</sub>1.3  $\alpha$  subunit protein, and comparing the presence of the protein in the control sample with the presence of the protein in the test sample.

The invention also includes kits for detecting the presence of mNa<sub>v</sub>1.3  $\alpha$  subunit proteins in a biological sample. For example, the kit can include a compound or agent capable of detecting mNa<sub>v</sub>1.3  $\alpha$  subunit protein or mRNA in a biological sample; and a standard. The compound or agent can be packaged in a suitable container. The kit can further comprise instructions for using the kit to detect mNa<sub>v</sub>1.3  $\alpha$  subunit proteins or nucleic acids.

For antibody-based kits, the kit can include: (1) a first antibody (e.g., attached to a solid support) which binds to a polypeptide corresponding to a marker of the invention; and, optionally, (2) a second, different antibody which binds to either the polypeptide or the first antibody and is conjugated to a detectable agent.

For oligonucleotide-based kits, the kit can include: (1) an oligonucleotide, e.g., a detectably labeled oligonucleotide, which hybridizes to a nucleic acid sequence encoding a polypeptide corresponding to a marker of the invention or (2) a pair of primers useful for amplifying a nucleic acid molecule corresponding to a marker of the invention. The kit can also include a buffering agent, a preservative, or a protein stabilizing agent. The kit can also include components necessary for detecting the detectable agent (e.g., an enzyme or a substrate). The kit can also contain a control sample or a series of control samples which can be assayed and compared to the test sample contained. Each component of the kit can be enclosed within an individual container and all of the various containers can be within a single package, along with instructions for interpreting the results of the assays performed using the kit.

The diagnostic methods described herein can identify subjects having, or at risk of developing, a disease or disorder associated with Na<sup>+</sup> channel expression or activity. As used herein, the term "unwanted" includes an unwanted phenomenon involved in a biological response such as neuropathic pain.

In one embodiment, a disease or disorder associated with Na<sup>+</sup> channel expression or activity is identified. A test sample is obtained from a subject and one or more Na<sup>+</sup> channel proteins or nucleic acids (e.g., mRNA or genomic DNA) are evaluated, wherein the level, e.g.,

the presence or absence, of a  $\text{Na}^+$  channel protein or nucleic acid is diagnostic for a subject having or at risk of developing a disease or disorder associated with  $\text{Na}^+$  channel expression or activity. As used herein, a "test sample" refers to a biological sample obtained from a subject of interest, including a biological fluid (e.g., serum), cell sample, or tissue, e.g., brain tissue.

5 The prognostic assays described herein can be used to determine whether a subject can be administered an agent (e.g., an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate) to treat a disease or disorder associated with  $\text{mNa}_v1.3 \alpha$  subunit expression or activity. For example, such methods can be used to determine whether a subject can be effectively treated with an agent for a pain disorder or for traumatic  
10 brain injury.

In another aspect, the invention features a computer medium having a plurality of digitally encoded data records. Each data record includes a value representing the level of expression or activity of a  $\text{mNa}_v1.3 \alpha$  subunit channel in a sample, and a descriptor of the sample. The descriptor of the sample can be an identifier of the sample, a compound which with  
15 the sample was treated, a subject from which the sample was derived (e.g., a patient), a diagnosis, or a treatment (e.g., a preferred treatment). In a preferred embodiment, the data record further includes values representing the level of expression of genes other than the  $\text{mNa}_v1.3 \alpha$  subunit channel (e.g., other genes associated with a disorder related to activity of the  $\text{mNa}_v1.3 \alpha$  subunit channel, or other genes on an array). The data record can be structured as a  
20 table, e.g., a table that is part of a database such as a relational database (e.g., a SQL database of the Oracle or Sybase database environments).

Also featured is a method of evaluating a sample. The method includes providing a sample, e.g., from the subject, and determining a gene expression profile of the sample, wherein  
25 the profile includes a value representing the level of  $\text{mNa}_v1.3 \alpha$  subunit channel expression or activity. The method can further include comparing the value or the profile (i.e., multiple values) to a reference value or reference profile. The gene expression profile of the sample can be obtained by any of the methods described herein (e.g., by providing a nucleic acid from the sample and contacting the nucleic acid to an array, or by assaying the activity of a  $\text{mNa}_v1.3 \alpha$  subunit channel in the sample). The method can be used to diagnose a disorder in a subject  
30 wherein a change in  $\text{mNa}_v1.3 \alpha$  subunit expression is an indication that the subject has or is disposed to having a disorder. The method can be used to monitor a treatment, e.g., for pain in a

subject. For example, the gene expression profile can be determined for a sample from a subject undergoing treatment. The profile can be compared to a reference profile or to a profile obtained from the subject prior to treatment or prior to onset of the disorder (see, e.g., Golub *et al.*, *Science* 286:531, 1999).

5 In yet another aspect, the invention features a method of evaluating a test compound (see also, "Screening Assays", above). The method includes providing a cell and a test compound; contacting the test compound to the cell; obtaining a subject expression profile for the contacted cell; and comparing the subject expression profile to one or more reference profiles. The profiles include a value representing the level of mNa<sub>y</sub>1.3  $\alpha$  subunit activity or expression. In a preferred 10 embodiment, the subject activity or expression profile is compared to a target profile, e.g., a profile for a normal cell or for a desired condition of a cell. The test compound is evaluated favorably if the subject expression profile is more similar to the target profile than an expression profile obtained from an uncontacted cell.

15 In another aspect, the invention features, a method of evaluating a subject. The method includes: a) obtaining a sample from a subject, e.g., from a caregiver, e.g., a caregiver who obtains the sample from the subject; b) determining a subject expression profile for the sample. Optionally, the method further includes either or both of steps: c) comparing the subject expression profile to one or more reference expression profiles; and d) selecting the reference profile most similar to the subject reference profile. The subject expression profile and the 20 reference profiles include a value representing the level of mNa<sub>y</sub>1.3  $\alpha$  subunit activity or expression. A variety of routine statistical measures can be used to compare two reference profiles. One possible metric is the length of the distance vector that is the difference between the two profiles. Each of the subject and reference profile is represented as a multi-dimensional vector, wherein each dimension is a value in the profile.

25 The method can further include transmitting a result to a caregiver. The result can be the subject expression profile, a result of a comparison of the subject expression profile with another profile, a most similar reference profile, or a descriptor of any of the aforementioned. The result can be transmitted across a computer network, e.g., the result can be in the form of a computer transmission, e.g., a computer data signal embedded in a carrier wave.

30 Also featured is a computer medium having executable code for effecting the following steps: receive a subject expression profile (e.g., any subject expression profile described herein);

access a database of reference expression profiles; and either i) select a matching reference profile most similar to the subject expression profile or ii) determine at least one comparison score for the similarity of the subject expression profile to at least one reference profile. The subject expression profile, and the reference expression profiles each include a value

5 representing the level of mNa<sub>v</sub>1.3  $\alpha$  subunit activity or expression.

Arrays and Uses Thereof

In another aspect, the invention features an array that includes a substrate having a plurality of addresses. At least one address of the plurality includes a capture probe that binds specifically to a molecule corresponding to a Na<sup>+</sup> channel a subunit, e.g., a mNa<sub>v</sub>1.3  $\alpha$  subunit 10 nucleic acid or polypeptide. The array can have a density of at least 10, 100, 1,000, or 10,000 or more addresses/cm<sup>2</sup>, and ranges between. The substrate can be a two-dimensional substrate such as a glass slide, a wafer (e.g., silica or plastic), a mass spectroscopy plate, or a three-dimensional substrate such as a gel pad. Addresses in addition to address of the plurality can be disposed on the array.

15 In a preferred embodiment, at least one address of the plurality includes a nucleic acid capture probe that hybridizes specifically to a mNa<sub>v</sub>1.3  $\alpha$  subunit nucleic acid, e.g., the sense or anti-sense strand. A subset of addresses of the plurality of addresses can be a nucleic acid capture probe for a Na<sup>+</sup> channel gene encoding a mNa<sub>v</sub>1.3  $\alpha$  subunit. Each address of the subset 20 can include a capture probe that hybridizes to a different region of a mNa<sub>v</sub>1.3  $\alpha$  subunit nucleic acid. The array can be used to sequence the gene by hybridization (see, e.g., U.S. Patent No. 5,695,940).

An array can be generated by various methods, e.g., by photolithographic methods (see, e.g., U.S. Patent Nos. 5,143,854; 5,510,270; and 5,527,681), mechanical methods (e.g., directed-flow methods as described in U.S. Patent No. 5,384,261), pin-based methods (e.g., as described 25 in U.S. Pat. No. 5,288,514), and bead-based techniques (e.g., as described in PCT US/93/04145).

In another embodiment, at least one address of the plurality includes a polypeptide capture probe that binds specifically to a mNa<sub>v</sub>1.3  $\alpha$  subunit polypeptide or fragment thereof. The polypeptide can be a naturally-occurring interaction partner of a mNa<sub>v</sub>1.3  $\alpha$  subunit 30 polypeptide. Preferably, the polypeptide is an antibody, e.g., an antibody described herein (see "Anti-mNa<sub>v</sub>1.3  $\alpha$  subunit Antibodies,"), such as a monoclonal antibody or a single-chain antibody.

In another aspect, the invention features a method of analyzing the expression of mNa<sub>v</sub>1.3  $\alpha$  subunit. The method includes providing an array as described above; contacting the array with a sample and detecting binding of mNa<sub>v</sub>1.3  $\alpha$  subunit molecule (e.g., nucleic acid or polypeptide) to the array. Optionally the method further includes amplifying nucleic acid from the sample prior or during contact with the array.

In another embodiment, the array can be used to assay gene expression in a tissue to ascertain tissue specificity of genes in the array, particularly the expression of mNa<sub>v</sub>1.3  $\alpha$  subunits. If a sufficient number of diverse samples is analyzed, clustering (e.g., hierarchical clustering, k-means clustering, Bayesian clustering and the like) can be used to identify other genes which are co-regulated with mNa<sub>v</sub>1.3  $\alpha$  subunits. For example, the array can be used for the quantitation of the expression of multiple genes. Thus, not only tissue specificity, but also the level of expression of a battery of genes in the tissue is ascertained. Quantitative data can be used to group (e.g., cluster) genes on the basis of their tissue expression *per se* and level of expression in that tissue.

For example, array analysis of gene expression can be used to assess the effect of cell-cell interactions on mNa<sub>v</sub>1.3  $\alpha$  subunit expression. A first tissue can be perturbed and nucleic acid from a second tissue that interacts with the first tissue can be analyzed. In this context, the effect of one cell type on another cell type in response to a biological stimulus can be determined, e.g., to monitor the effect of cell-cell interaction at the level of gene expression.

In another embodiment, cells are contacted with a therapeutic agent. The expression profile of the cells is determined using the array, and the expression profile is compared to the profile of like cells not contacted with the agent. For example, the assay can be used to determine or analyze the molecular basis of an undesirable effect of the therapeutic agent. If an agent is administered therapeutically to treat one cell type but has an undesirable effect on another cell type, the invention provides an assay to determine the molecular basis of the undesirable effect and thus provides the opportunity to co-administer a counteracting agent or otherwise treat the undesired effect. Similarly, even within a single cell type, undesirable biological effects can be determined at the molecular level. Thus, the effects of an agent on expression of other than the target gene can be ascertained and counteracted.

In another embodiment, the array can be used to monitor expression of one or more genes in the array with respect to time. For example, samples obtained from different time points can

be probed with the array. Such analysis can identify and/or characterize the development of a disease or disorder associated with  $\text{Na}^+$  channel activity. The method can also evaluate the treatment and/or progression of a  $\text{Na}^+$  channel-associated disease or disorder

5 The array is also useful for ascertaining differential expression patterns of one or more genes in normal and abnormal cells. This provides a battery of genes (e.g., including genes encoding m $\text{Na}_v$ 1.3  $\alpha$  subunits) that could serve as a molecular target for diagnosis or therapeutic intervention.

In another aspect, the invention features an array having a plurality of addresses. Each address of the plurality includes a unique polypeptide. At least one address of the plurality has 10 disposed thereon a m $\text{Na}_v$ 1.3  $\alpha$  subunit polypeptide or fragment thereof. Methods of producing polypeptide arrays are described in the art, e.g., in De Wildt *et al.*, *Nature Biotech.* 18: 989-994, 2000; Lueking *et al.*, *Anal. Biochem.* 270:103-111, 1999; Ge, H., *Nucleic Acids Res.* 28:e3, 1-VII, 2000; MacBeath, G., and Schreiber, S.L., *Science* 289:1760-1763, 2000; and WO 15 99/51773A1. In a preferred embodiment, each addresses of the plurality has disposed thereon a polypeptide at least 60%-99 % identical to a m $\text{Na}_v$ 1.3  $\alpha$  subunit polypeptide or fragment thereof. For example, multiple variants of a m $\text{Na}_v$ 1.3  $\alpha$  subunit polypeptide (e.g., encoded by allelic variants, site-directed mutants, random mutants, or combinatorial mutants) can be disposed at individual addresses of the plurality.

20 The polypeptide array can be used to detect a  $\text{Na}^+$ -binding compound, e.g., an antibody in a sample from a subject with specificity for a m $\text{Na}_v$ 1.3  $\alpha$  subunit polypeptide or the presence of a  $\text{Na}^+$  channel-binding protein or ligand.

In another aspect, the invention features a method of analyzing a plurality of probes. The 25 method is useful, e.g., for analyzing gene expression. The method includes: providing a two-dimensional array having a plurality of addresses, each address of the plurality being positionally distinguishable from each other address of the plurality having a unique capture probe, e.g., wherein the capture probes are from a cell or subject which express  $\text{Na}^+$  channels comprising m $\text{Na}_v$ 1.3  $\alpha$  subunits or from a cell or subject in which a  $\text{Na}^+$  channel-mediated response has been elicited, e.g., by contact of the cell with  $\text{Na}^+$  channel m $\text{Na}_v$ 1.3  $\alpha$  subunit nucleic acids or proteins, or administration to the cell or subject  $\text{Na}^+$  channel m $\text{Na}_v$ 1.3  $\alpha$  subunit nucleic acids or proteins; providing a two dimensional array having a plurality of addresses, each address of the plurality being positionally distinguishable from each other address of the plurality, and each

address of the plurality having a unique capture probe, e.g., wherein the capture probes are from a cell or subject which does not express  $\text{Na}^+$  channel  $\text{mNa}_v1.3 \alpha$  subunits (or does not express as highly as in the case of the  $\text{Na}^+$  channel  $\text{mNa}_v1.3 \alpha$  subunit-positive plurality of capture probes) or from a cell or subject which in which a  $\text{Na}^+$  channel-mediated response has not been elicited (or has been elicited to a lesser extent than in the first sample); contacting the array with one or more inquiry probes (which is preferably other than a  $\text{Na}^+$  channel  $\text{mNa}_v1.3 \alpha$  subunit nucleic acid, polypeptide, or antibody), and thereby evaluating the plurality of capture probes. Binding, e.g., in the case of a nucleic acid, hybridization with a capture probe at an address of the plurality, is detected, e.g., by signal generated from a label attached to the nucleic acid, polypeptide, or antibody.

In another aspect, the invention features a method of analyzing  $\text{mNa}_v1.3 \alpha$  subunits, e.g., analyzing structure, function, or relatedness to other nucleic acid or amino acid sequences. The method includes: providing a  $\text{mNa}_v1.3 \alpha$  subunit nucleic acid or amino acid sequence; comparing the sequence(s) with one or more preferably a plurality of sequences from a collection of sequences, e.g., a nucleic acid or protein sequence database; to thereby analyze  $\text{mNa}_v1.3 \alpha$  subunit subunits.

#### *Detection Assays*

Portions or fragments of the cDNA sequences identified herein (and the corresponding complete gene sequences) can be used in numerous ways as polynucleotide reagents.

For example, polynucleotide reagents can be used for diagnostic assays, prognostic assays, and monitoring clinical trials are used for prognostic (predictive) purposes to thereby treat an individual prophylactically. Accordingly, one aspect of the present invention relates to diagnostic assays for determining  $\text{mNa}_v1.3 \alpha$  subunit protein and/or nucleic acid expression as well as  $\text{mNa}_v1.3 \alpha$  subunit activity, in the context of a biological sample (e.g., blood, serum, cells, tissue) to thereby determine whether an individual is afflicted with a disease or disorder, or is at risk of developing a disorder, associated with aberrant or unwanted  $\text{mNa}_v1.3 \alpha$  subunit expression or activity. The invention also provides for prognostic (or predictive) assays for determining whether an individual is at risk of developing a disorder associated with  $\text{mNa}_v1.3 \alpha$  subunit protein, nucleic acid expression or activity. For example, mutations in a gene encoding a

m<sub>Na</sub>,1.3  $\alpha$  subunit can be assayed in a biological sample, and used for prognostic or predictive purposes.

Another aspect of the invention pertains to monitoring the influence of agents (e.g., drugs, compounds) on the expression or activity of m<sub>Na</sub>,1.3  $\alpha$  subunit *in vivo*.

5 Monitoring the influence of agents (e.g., drugs) on the expression or activity of a m<sub>Na</sub>,1.3  $\alpha$  subunit protein (e.g., the modulation of membrane excitability) can be applied not only in basic drug screening, but also in clinical trials. For example, the effectiveness of an agent determined by a screening assay as described herein to increase m<sub>Na</sub>,1.3  $\alpha$  subunit gene expression, protein levels, or upregulate m<sub>Na</sub>,1.3  $\alpha$  subunit activity, can be monitored in clinical 10 trials of subjects exhibiting decreased or increased m<sub>Na</sub>,1.3  $\alpha$  subunit gene expression, protein levels, or downregulated m<sub>Na</sub>,1.3  $\alpha$  subunit. Other genes that have been implicated in, for example, a Na<sup>+</sup> channel associated disorder can be used markers of the phenotype of a particular cell.

15 *Therapeutic Methods*

Another aspect of the invention pertains to methods of modulating m<sub>Na</sub>,1.3  $\alpha$  subunit expression or activity for therapeutic purposes. Accordingly, in an exemplary embodiment, the modulatory method of the invention involves contacting a cell with a m<sub>Na</sub>,1.3  $\alpha$  subunit or agent that modulates one or more of the activities of m<sub>Na</sub>,1.3  $\alpha$  subunit protein activity associated 20 with the cell. An agent that modulates m<sub>Na</sub>,1.3  $\alpha$  subunit protein activity can be an agent as described herein, such as a nucleic acid or a protein, a naturally-occurring target molecule of a m<sub>Na</sub>,1.3  $\alpha$  subunit protein (e.g., a m<sub>Na</sub>,1.3  $\alpha$  subunit substrate), a m<sub>Na</sub>,1.3  $\alpha$  subunit antibody, a m<sub>Na</sub>,1.3  $\alpha$  subunit agonist or antagonist, a peptidomimetic of a m<sub>Na</sub>,1.3  $\alpha$  subunit agonist or antagonist, or other small molecule. In one embodiment, the agent stimulates one or more 25 m<sub>Na</sub>,1.3  $\alpha$  subunit activities. Examples of such stimulatory agents include active m<sub>Na</sub>,1.3  $\alpha$  subunit protein and a nucleic acid molecule encoding m<sub>Na</sub>,1.3  $\alpha$  subunit that has been introduced into the cell. In another embodiment, the agent inhibits one or more m<sub>Na</sub>,1.3  $\alpha$  subunit activities. Examples of such inhibitory agents include antisense m<sub>Na</sub>,1.3  $\alpha$  subunit nucleic acid molecules, anti-m<sub>Na</sub>,1.3  $\alpha$  subunit antibodies, and m<sub>Na</sub>,1.3  $\alpha$  subunit inhibitors. 30 These modulatory methods can be performed *in vitro* (e.g., by culturing the cell with the agent)

or, alternatively, *in vivo* (e.g., by administering the agent to a subject). As such, the present invention provides methods of treating an individual afflicted with a disease or disorder characterized by aberrant or unwanted expression or activity of a mNa<sub>v</sub>1.3  $\alpha$  subunit protein or nucleic acid molecule. In one embodiment, the method involves administering an agent (e.g., an agent identified by a screening assay described herein), or combination of agents that modulates (e.g., upregulates or downregulates) mNa<sub>v</sub>1.3  $\alpha$  subunit expression or activity. In another embodiment, the method involves administering a mNa<sub>v</sub>1.3  $\alpha$  subunit protein or nucleic acid molecule as therapy to compensate for reduced or aberrant mNa<sub>v</sub>1.3  $\alpha$  subunit expression or activity.

Stimulation of mNa<sub>v</sub>1.3  $\alpha$  subunit activity is desirable in situations in which mNa<sub>v</sub>1.3  $\alpha$  subunit is abnormally downregulated and/or in which increased mNa<sub>v</sub>1.3  $\alpha$  subunit activity is likely to have a beneficial effect. Antagonism of activity may also be desirable. For example, modulators may be desirable for treatment of pain, e.g., neuropathic pain, head trauma, and neurodegenerative diseases. Neurodegenerative diseases include multiple sclerosis, Alzheimer's Disease, Parkinson's Disease or other forms of dementia, amyotrophic lateral sclerosis, Down's Syndrome, Huntington chorea and spinal cerebellar degeneration. Modulation of Na<sub>v</sub>1.3 channels can be useful, e.g., in the treatment of various disorders accompanying cerebrovascular injury or trauma including cerebral hemorrhages such as hypertensive intracerebral hemorrhage and subarachnoid hemorrhage, transient cerebral ischemic attacks, cerebroarteriosclerosis and their sequela, as well as brain damages at the time of revivification after cardiac arrest, brain dysfunction prior to or after brain surgery, disorders of the nervous system due to hypoxia, hypoglycemia, brain or spinal damage, intoxication with drugs or gases, diabetes mellitus, administration of anti-cancer agents, alcohol and the like.

#### 25 *Pharmaceutical Compositions*

As used herein, the compounds of this invention, e.g., Na<sup>+</sup> channel modulators identified by the methods described herein, are defined to include pharmaceutically acceptable derivatives or prodrugs thereof. A "pharmaceutically acceptable derivative or prodrug" means any pharmaceutically acceptable salt, ester, salt of an ester, or other derivative of a compound of this invention which, upon administration to a recipient, is capable of providing (directly or indirectly) a compound of this invention. Particularly favored derivatives and prodrugs are those

that increase the bioavailability of the compounds of this invention when such compounds are administered to a mammal (e.g., by allowing an orally administered compound to be more readily absorbed into the blood) or which enhance delivery of the parent compound to a biological compartment (e.g., the brain or lymphatic system) relative to the parent species.

5 Preferred prodrugs include derivatives where a group which enhances aqueous solubility or active transport through the gut membrane is appended to the structure of formulae described herein.

10 The compounds of this invention may be modified by appending appropriate functionalities to enhance selective biological properties. Such modifications are known in the art and include those which increase biological penetration into a given biological compartment (e.g., blood, lymphatic system, central nervous system), increase oral availability, increase 15 solubility to allow administration by injection, alter metabolism and alter rate of excretion.

15 Pharmaceutically acceptable salts of the compounds of this invention include those derived from pharmaceutically acceptable inorganic and organic acids and bases. Examples of suitable acid salts include acetate, adipate, alginate, aspartate, benzoate, benzenesulfonate, 20 bisulfate, butyrate, citrate, camphorate, camphorsulfonate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptanoate, glycolate, hemisulfate, heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, lactate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, palmoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, salicylate, 25 succinate, sulfate, tartrate, thiocyanate, tosylate and undecanoate. Other acids, such as oxalic, while not in themselves pharmaceutically acceptable, may be employed in the preparation of salts useful as intermediates in obtaining the compounds of the invention and their pharmaceutically acceptable acid addition salts. Salts derived from appropriate bases include alkali metal (e.g., sodium), alkaline earth metal (e.g., magnesium), ammonium and N-(alkyl)<sub>4</sub><sup>+</sup> salts. This invention also envisions the quaternization of any basic nitrogen-containing groups of the compounds disclosed herein. Water or oil-soluble or dispersible products may be obtained by such quaternization. Salt forms of the compounds of any of the formulae herein can be amino acid salts of carboxy groups (e.g. L-arginine, -lysine, -histidine salts).

30 The compounds of the formulae described herein can, for example, be administered by injection, intravenously, intraarterially, subdermally, intraperitoneally, intramuscularly, or

subcutaneously; or orally, buccally, nasally, transmucosally, topically, in an ophthalmic preparation, or by inhalation, with a dosage ranging from about 0.5 to about 100 mg/kg of body weight, alternatively dosages between 1 mg and 1000 mg/dose, every 4 to 120 hours, or according to the requirements of the particular drug. The methods herein contemplate 5 administration of an effective amount of compound or compound composition to achieve the desired or stated effect. Typically, the pharmaceutical compositions of this invention will be administered from about 1 to about 6 times per day or alternatively, as a continuous infusion. Such administration can be used as a chronic or acute therapy. The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form will vary 10 depending upon the host treated and the particular mode of administration. A typical preparation will contain from about 5% to about 95% active compound (w/w). Alternatively, such preparations contain from about 20% to about 80% active compound.

Lower or higher doses than those recited above may be required. Specific dosage and treatment regimens for any particular patient will depend upon a variety of factors, including the 15 activity of the specific compound employed, the age, body weight, general health status, sex, diet, time of administration, rate of excretion, drug combination, the severity and course of the disease, condition or symptoms, the patient's disposition to the disease, condition or symptoms, and the judgment of the treating physician.

Upon improvement of a patient's condition, a maintenance dose of a compound, 20 composition or combination of this invention may be administered, if necessary. Subsequently, the dosage or frequency of administration, or both, may be reduced, as a function of the symptoms, to a level at which the improved condition is retained when the symptoms have been alleviated to the desired level. Patients may, however, require intermittent treatment on a long-term basis upon any recurrence of disease symptoms.

25 The compositions delineated herein include the compounds of the formulae delineated herein, as well as additional therapeutic agents if present, in amounts effective for achieving a modulation of disease or disease symptoms, including ion channel-mediated disorders or symptoms thereof.

The term "pharmaceutically acceptable carrier or adjuvant" refers to a carrier or adjuvant 30 that may be administered to a patient, together with a compound of this invention, and which

does not destroy the pharmacological activity thereof and is nontoxic when administered in doses sufficient to deliver a therapeutic amount of the compound.

Pharmaceutically acceptable carriers, adjuvants and vehicles that may be used in the pharmaceutical compositions of this invention include, but are not limited to, ion exchangers, alumina, aluminum stearate, lecithin, self-emulsifying drug delivery systems (SEDDS) such as d- $\alpha$ -tocopherol polyethyleneglycol 1000 succinate, surfactants used in pharmaceutical dosage forms such as Tweens or other similar polymeric delivery matrices, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes, polyethylene-polyoxypropylene-block polymers, polyethylene glycol and wool fat. Cyclodextrins such as  $\alpha$ -,  $\beta$ -, and  $\gamma$ -cyclodextrin, or chemically modified derivatives such as hydroxyalkylcyclodextrins, including 2- and 3-hydroxypropyl- $\beta$ -cyclodextrins, or other solubilized derivatives may also be advantageously used to enhance delivery of compounds of the formulae described herein.

The pharmaceutical compositions of this invention may be administered orally, parenterally, by inhalation spray, topically, rectally, nasally, buccally, vaginally or via an implanted reservoir, preferably by oral administration or administration by injection. The pharmaceutical compositions of this invention may contain any conventional non-toxic pharmaceutically-acceptable carriers, adjuvants or vehicles. In some cases, the pH of the formulation may be adjusted with pharmaceutically acceptable acids, bases or buffers to enhance the stability of the formulated compound or its delivery form. The term parenteral as used herein includes subcutaneous, intracutaneous, intravenous, intramuscular, intraarticular, intraarterial, intrasynovial, intrasternal, intrathecal, intralesional and intracranial injection or infusion techniques.

The pharmaceutical compositions may be in the form of a sterile injectable preparation, for example, as a sterile injectable aqueous or oleaginous suspension. This suspension may be formulated according to techniques known in the art using suitable dispersing or wetting agents (such as, for example, Tween 80) and suspending agents. The sterile injectable preparation may

also be a sterile injectable solution or suspension in a non-toxic parenterally acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are mannitol, water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil may be employed including synthetic mono- or diglycerides. Fatty acids, such as oleic acid and its glyceride derivatives are useful in the preparation of injectables, as are natural pharmaceutically-acceptable oils, such as olive oil or castor oil, especially in their polyoxyethylated versions. These oil solutions or suspensions may also contain a long-chain alcohol diluent or dispersant, or carboxymethyl cellulose or similar dispersing agents which are commonly used in the formulation of pharmaceutically acceptable dosage forms such as emulsions and or suspensions. Other commonly used surfactants such as Tweens or Spans and/or other similar emulsifying agents or bioavailability enhancers which are commonly used in the manufacture of pharmaceutically acceptable solid, liquid, or other dosage forms may also be used for the purposes of formulation.

15 The pharmaceutical compositions of this invention may be orally administered in any orally acceptable dosage form including, but not limited to, capsules, tablets, emulsions and aqueous suspensions, dispersions and solutions. In the case of tablets for oral use, carriers which are commonly used include lactose and corn starch. Lubricating agents, such as magnesium stearate, are also typically added. For oral administration in a capsule form, useful diluents include lactose and dried cornstarch. When aqueous suspensions and/or emulsions are administered orally, the active ingredient may be suspended or dissolved in an oily phase is combined with emulsifying and/or suspending agents. If desired, certain sweetening and/or flavoring and/or coloring agents may be added.

20 The pharmaceutical compositions of this invention may also be administered in the form of suppositories for rectal administration. These compositions can be prepared by mixing a compound of this invention with a suitable non-irritating excipient which is solid at room temperature but liquid at the rectal temperature and therefore will melt in the rectum to release the active components. Such materials include, but are not limited to, cocoa butter, beeswax and polyethylene glycols.

25 Topical administration of the pharmaceutical compositions of this invention is useful when the desired treatment involves areas or organs readily accessible by topical application. For

application topically to the skin, the pharmaceutical composition should be formulated with a suitable ointment containing the active components suspended or dissolved in a carrier. Carriers for topical administration of the compounds of this invention include, but are not limited to, mineral oil, liquid petroleum, white petroleum, propylene glycol, polyoxyethylene

5 polyoxypropylene compound, emulsifying wax and water. Alternatively, the pharmaceutical composition can be formulated with a suitable lotion or cream containing the active compound suspended or dissolved in a carrier with suitable emulsifying agents. Suitable carriers include, but are not limited to, mineral oil, sorbitan monostearate, polysorbate 60, cetyl esters wax, cetaryl alcohol, 2-octyldodecanol, benzyl alcohol and water. The pharmaceutical compositions 10 of this invention may also be topically applied to the lower intestinal tract by rectal suppository formulation or in a suitable enema formulation. Topically-transdermal patches are also included in this invention.

The pharmaceutical compositions of this invention may be administered by nasal aerosol or inhalation. Such compositions are prepared according to techniques well-known in the art of 15 pharmaceutical formulation and may be prepared as solutions in saline, employing benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, fluorocarbons, and/or other solubilizing or dispersing agents known in the art.

A composition having the compound of the formulae herein and an additional agent (e.g., a therapeutic agent) can be administered using an implantable device. Implantable devices and 20 related technology are known in the art and are useful as delivery systems where a continuous, or timed-release delivery of compounds or compositions delineated herein is desired. Additionally, the implantable device delivery system is useful for targeting specific points of compound or composition delivery (e.g., localized sites, organs). Negrin et al., *Biomaterials*, 22(6):563 (2001). Timed-release technology involving alternate delivery methods can also be used in this 25 invention. For example, timed-release formulations based on polymer technologies, sustained-release techniques and encapsulation techniques (e.g., polymeric, liposomal) can also be used for delivery of the compounds and compositions delineated herein.

Also within the invention is a patch to deliver active chemotherapeutic combinations herein. A patch includes a material layer (e.g., polymeric, cloth, gauze, bandage) and the 30 compound of the formulae herein as delineated herein. One side of the material layer can have a protective layer adhered to it to resist passage of the compounds or compositions. The patch can

5 additionally include an adhesive to hold the patch in place on a subject. An adhesive is a composition, including those of either natural or synthetic origin, that when contacted with the skin of a subject, temporarily adheres to the skin. It can be water resistant. The adhesive can be placed on the patch to hold it in contact with the skin of the subject for an extended period of  
10 time. The adhesive can be made of a tackiness, or adhesive strength, such that it holds the device in place subject to incidental contact, however, upon an affirmative act (e.g., ripping, peeling, or other intentional removal) the adhesive gives way to the external pressure placed on the device or the adhesive itself, and allows for breaking of the adhesion contact. The adhesive can be pressure sensitive, that is, it can allow for positioning of the adhesive (and the device to be  
15 adhered to the skin) against the skin by the application of pressure (e.g., pushing, rubbing,) on the adhesive or device.

When the compositions of this invention comprise a combination of a compound of the formulae described herein and one or more additional therapeutic or prophylactic agents, both the compound and the additional agent should be present at dosage levels of between about 1 to 100%, and more preferably between about 5 to 95% of the dosage normally administered in a  
15 monotherapy regimen. The additional agents may be administered separately, as part of a multiple dose regimen, from the compounds of this invention. Alternatively, those agents may be part of a single dosage form, mixed together with the compounds of this invention in a single composition.

20 The compounds and methods described above can be used for the therapeutic modulation of  $\text{Na}^+$  channel function.

#### **Example 1 : Identification of a novel $\text{Na}^+$ channel $\alpha$ subunit**

1. First, cDNA was generated from mouse brain polyA RNA (Stratagene and Clontech)  
25 using SuperScript First-Strand Synthesis System for RT-PCR (Invitrogen). Primers were designed for amplification of a mouse  $\text{Na}_v1.3$   $\alpha$  subunit. A cDNA corresponding to a partial sequence of a mouse  $\text{Na}_v1.3$   $\alpha$  subunit (GenBank® Acc. No. NM\_018732) aligns with the middle of the published rat cDNA sequence (NM\_013119; at nucleotide 2461 from the initiation codon). To identify the full length sequence for mouse  $\text{Na}_v1.3$   $\alpha$  subunit, the rat sequence  
30 (NM\_013199) was compared to the mouse genome using BLAST® (Blake JA, et al. MGD: The Mouse Genome Database. *Nucleic Acids Res* 31: 193-195, 2003).

2. A 5' primer was designed to anneal 7 nucleotides upstream of the putative initiation codon and a 3' primer was designed to anneal 124 nucleotides downstream of the putative stop codon. Each primer contained a NotI restriction site at the end. Primers were designed to anneal 5 to the untranslated regions. Primers that anneal to untranslated regions are less likely to amplify non-Na<sub>v</sub>1.3  $\alpha$  subunit genes, i.e., mouse Na<sub>v</sub>1.1 and Na<sub>v</sub>1.2  $\alpha$  subunit genes. The 3' primer that was used to amplify mNa<sub>v</sub>1.3 was designed to anneal in an untranslated region corresponding to a region of high variability between rat Na<sub>v</sub>1.1, Na<sub>v</sub>1.2, and Na<sub>v</sub>1.3  $\alpha$  subunit 10 3' untranslated regions to increase specificity for mNa<sub>v</sub>1.3. In this region in rat Na<sub>v</sub>1  $\alpha$  subunit genes, rat Na<sub>v</sub>1.1 differs from rat Na<sub>v</sub>1.2 by 17 out of 32 nucleotides, and rat Na<sub>v</sub>1.2 differs from rat Na<sub>v</sub>1.3 by 13 out of 32 nucleotides. This untranslated region also has a high degree of homology between rat 15 and murine sequences; only 29 of 32 nucleotides are conserved.

3. Cloning of a mouse Na<sub>v</sub>1.3  $\alpha$  subunit

15 cDNA encoding a mouse Na<sub>v</sub>1.3  $\alpha$  subunit was amplified from mouse brain cDNA using Herculase Hotstart DNA Polymerase (Stratagene; 30 cycles). The PCR product was resolved by agarose gel electrophoresis and extracted from the gel using Qiaquick Gel Extraction kit (Qiagen). The extracted product was subcloned into pCR-XL-TOPO vector using the TOPO XL PCR Cloning Kit (Invitrogen). The resulting vectors were transformed into XL10 Gold 20 Ultracompetent Cells (Stratagene). Transformed bacteria were cultured. DNA was isolated from the transformed bacteria using an Aurum Miniprep (Biorad).

25 Clones containing a mouse Na<sub>v</sub>1.3  $\alpha$  subunit insert were identified by agarose gel analysis of uncut DNA and NotI (New England Biolabs) digested DNA. Sequence analysis of the 5' and 3' ends of two clones (A and B; SeqWright) was performed to verify the presence of the mouse Na<sub>v</sub>1.3  $\alpha$  subunit insert

Bacteria containing the clones A and B were cultured for preparation of a larger quantity 30 of DNA by maxi prep (Qiagen).

4. Full length sequencing of clones A and B (SeqWright) was performed. To determine if 30 PCR errors were present in clones A and B, the nucleotide sequence of the two clones were compared to each other, to the mouse Na<sub>v</sub>1.3  $\alpha$  subunit genomic sequence, to the sequence of the

rat  $\text{Na}_v1.3 \alpha$  subunit cDNA (NM\_013199) and to two human  $\text{Na}_v1.3 \alpha$  subunit cDNAs (NM\_006922 and AF225986). In addition, the predicted amino acid sequences encoded by nucleotide sequence of clones A and B were generated and compared with the rat and human  $\text{Na}_v1.3 \alpha$  subunit protein sequences.

5 Clone A contained one nucleotide change relative to the murine genomic sequence and a human  $\text{Na}_v1.3 \alpha$  subunit sequence. Clone A also contained an 83 nucleotide deletion relative to clone B.

10 Clone A and clone B both contained an extra A in a string of six As which was most likely introduced by PCR error. The presence of this nucleotide in a coding sequence would shift the reading frame and encode a protein that is 1504 amino acids (as opposed to approximately 1940 amino acids in known  $\text{Na}_v \alpha$  subunit polypeptides). Also, the extra A is not present in the genomic murine sequence. The rat and human sequences also lack an extra nucleotide at that position.

15 Clone B contained two nucleotide changes that were not present in clone A or corresponding genomic regions in murine, rat, and human genomic sequences, and thus were determined to be errors. Clone B also had an extra nucleotide that was not present in clone A or in the corresponding position in murine, rat, and human genomic sequences, which was also determined to be an error.

20 5. Repair of the mutations found in the mouse  $\text{Na}_v1.3 \alpha$  subunit clones has been performed with the following steps. For step 1 of the repair, the mutation and the 83 nucleotide deletion in clone A were corrected by replacing the mutated fragment with the corresponding fragment from clone B as follows. Clones A and B were digested with XcmI (New England Biolabs). The large fragment of clone A (which lacks the mutation and deletion) and the small fragment of 25 clone B (to replace the fragment from clone A) were resolved and extracted from an agarose gel using a Qiaquick Gel Extraction kit. The digested fragments were ligated using T4 DNA ligase (New England Biolabs). The ligated product was transformed into MAX Efficiency Stbl2 Competent Cells (Invitrogen). Bacteria were cultured and DNA was isolated with an Aurum miniprep. Clones containing the appropriate XcmI fragment were identified by agarose gel analysis of uncut DNA and DNA digested with SmaI and NarI (New England Biolabs).

Sequence analysis of several clones (SeqWright) was performed to identify DNA in which the mutation and 83 nucleotide deletion was repaired and to verify the presence of the two extra nucleotides (one extra nucleotide was present in clone A and one was carried over with the XcmI fragment from clone B).

5

6. For step 2 of the repair of the isolated mouse  $\text{Na}_v1.3 \alpha$  subunit cDNA, a clone in which the mutation and 83 nucleotide deletion had been repaired was used. One extra nucleotide in this clone was corrected with a QuickChange XL Site-Directed Mutagenesis Kit (Stratagene). Site-directed mutagenesis primers were designed. The mutagenesis reaction was performed, and the

10 product of the reaction was transformed into XL10 Gold Ultracompetent Cells (Stratagene).

Transformed bacteria were cultured and DNA was isolated by Aurum miniprep. Clones were analyzed with SmaI digestion of DNA. Sequence analysis of several clones was performed to identify DNA in which the extra nucleotide was repaired. Bacteria from one corrected clone were regrown so the insert containing the extra nucleotide could be transferred to pcDNA6B and

15 repaired.

7. To transfer the mouse  $\text{Na}_v1.3 \alpha$  subunit cDNA that contained one extra nucleotide from pCR-XL-TOPO into pcDNA6B, the clone was digested with EcoRV and SpeI (New England

Biolabs). pcDNA6B was digested with EcoRV and XbaI (New England Biolabs). Digested

20 fragments were resolved and extracted from an agarose gel using a Quiaquick Gel Extraction kit and ligated using T4 DNA ligase. The ligated product was transformed into XL10 Gold Ultracompetent Cells. Bacteria were cultured and DNA was isolated with an Aurum miniprep.

Clones containing mouse  $\text{Na}_v1.3 \alpha$  subunit were identified by agarose gel analysis of uncut DNA and SmaI digested DNA. One clone was regrown for the final step of repair.

25

8. For step 3 of the repair process, a fragment containing the extra nucleotide in mouse  $\text{Na}_v1.3 \alpha$  subunit in pcDNA6B was replaced with a newly amplified fragment as follows.

Primers were designed to amplify an internal fragment of mouse  $\text{Na}_v1.3 \alpha$  subunit (nucleotide 3950-4675 from the initiation codon). The fragment was amplified from mouse brain cDNA

30 (generated in step 1) with PfuTurbo Hotstart DNA Polymerase (Stratagene), resolved by agarose gel electrophoresis and extracted from the agarose gel using a Quiaquick Gel Extraction kit. The

fragment was subcloned into a pCR-XL-TOPO vector and transformed into One Shot TOP10 Competent Cells (Invitrogen). Transformed bacteria were cultured and DNA was isolated by an Aurum miniprep. Clones containing the mouse  $\text{Na}_v1.3$   $\alpha$  subunit fragment were identified by agarose gel analysis of DNA digested with EcoRI and double digested with ScaI and BglII (New England Biolabs). Sequence analysis of several clones was performed to identify DNA clones lacking mutations. One clone was regrown.

Replacement of the fragment containing an extra nucleotide in mouse  $\text{Na}_v1.3$   $\alpha$  subunit in pcDNA6B with the corrected fragment from pCR-XL-TOPO was performed as follows. The two clones were digested with HpaI and BstEII (New England Biolabs). Digested fragments were resolved and extracted from an agarose gel using a Quiagquick Gel Extraction kit. The fragments were ligated using T4 DNA ligase and the ligated product was transformed into MAX Efficiency Stbl2 Competent Cells. Bacteria were cultured and DNA was isolated with an Aurum miniprep. Clones were analyzed by restriction digestion with HpaI, BstEII and SmaI. Sequencing of the "swapped" fragment (SeqWright) of several clones was performed to identify clones in which the extra nucleotide was repaired. Full length sequencing of a single clone was performed to verify that no new mutations were produced during the site-directed mutagenesis procedure (step 2 of repair).

#### **Example 2 : Expression of a novel $\text{Na}^+$ channel $\alpha$ subunit**

The functional properties of the mouse  $\text{Na}_v1.3$   $\alpha$  subunit isolated in Example 1 were analyzed in the *Xenopus* oocyte expression system using the two electrode voltage clamp technique. The  $\text{Na}_v1.3$   $\alpha$  subunit cDNA was amplified and subcloned into the pcDNA6 vector (Invitrogen) by standard cloning methods. Approximately forty-six nanoliters of the mouse  $\text{Na}_v1.3$   $\alpha$  subunit (2 ng/ $\mu$ l) cDNA was injected into defolliculated *Xenopus* oocytes and currents were measured 5 days later. Sodium currents from six cells were recorded using electrodes of 0.5-1.0 M $\Omega$  resistance (3 M KCl) in a solution of 1.8 mM CaCl<sub>2</sub>, 5 mM HEPES-Na, 2 mM KCl, 1 mM MgCl and 96 mM NaAc, pH 7.5. The oocytes were held at -100 mV and depolarized to voltages ranging from -80 mV to 50 mV. Data was acquired using a P/4 leak subtraction protocol. Figure 3 shows representative sodium currents at different depolarizing voltages. The normalized, averaged peak current-voltage relationship of the six cells is shown in Figure 4. The channels begin to open at -40 mV and maximum currents are reached at 0 mV. These data show

that the channel encoded by the  $\text{Na}_v1.3\alpha$  cDNA is functional and that it exhibits biophysical properties expected for this type of channel.

**Table 1. Nucleotide sequence of mouse  $\text{Na}_v1.3\alpha$  subunit**

5 SEQ ID NO:3 corresponds to the entire sequence shown in Table 1. SEQ ID NO:1 corresponds to the coding sequence portion of the sequence shown in Table 1. The bold, underlined ATG sequence at nucleotides 8-10 and the bold, underlined TGA sequence at nucleotides 5945-5947 represent the initial and final codons of the coding sequence, respectively.

10	1	TGAAAAGATG	GCCCCAGGCC	TGCTGGTAC	CCCGGGACCT	GAGAGCTTCC
	51	GCCTTTAC	TCGAAGAAC	CTTGGCTA	TGAAAAGACG	TGCTGCAAGAA
	101	GAGAAAGCCA	AGAAACCCAA	GAAAGAACAA	GACATTGACG	ATGAGAACAA
	151	GCCAAAGCCA	AACAGTGA	TGGAAGCTGG	GAAGAACCTT	CCATTATCT
15	201	ATGGAGACAT	TCCCTCCAGAG	ATGGTGTGG	AGCCTCTGGA	GGACCTGGAC
	251	CCCTACTAC	TCACTGAAGAA	AACTTTTGTA	GTGTTGAATA	AAGGGAAAGC
	301	AATTTTCTGA	TTCAGTGC	CCTCCGGCTT	GTATATT	ACTCCACTAA
	351	ACCCCTGAG	GAAAATGCT	ATTAAGATTT	TGGTACACT	TTTATTAGC
20	401	ATGCTTATCA	TGTGCACTAT	TTTGACCAAC	TGTTGATT	TGACATTGAG
	451	CAATCCTCT	GACTGGACGA	AGAAATGTA	GTACACATTC	ACTGGGATCT
	501	ATACCTTGTG	GTCACTATCA	AAAGATCTTG	CCAGAGGATT	CTGCTTAAAGA
	551	GATTTCA	TCTCTCGTGA	CCCATGGAA	TGGCTGGATT	TCACTGTCAT
	601	CGTGATGGCA	TATGTGACAG	AGTTTGTGGA	CCTGGGAAT	GTCTCAGCGC
	651	TGAGAACCTT	CAGAGTCTTC	CGAGCATTTGA	AAACAAATATC	AGTCATTCTCA
25	701	GGTTAAAGA	CCATCTGGG	GGCCCTGATC	CAGTCGGTGA	AGAACGCTGTC
	751	TGACGTATG	ATACTCACTG	TGTTCTGCT	GAGCGCTTT	GCTCTCATCG
	801	GGCTGCACT	CTTCATGGGC	AACCTGAGGA	ATAAATGCTT	GCAGTGGCCT
	851	CCAAGCGAT	CTGCTTTGA	GATCAACACT	ACTTCTACT	TCAATGGCAC
	901	AATGGACTCA	AATGGGACAT	TTGTTAATG	AAACATGAGC	ACATTCAACT
30	951	GGAGGACTA	TATCGCAGAT	GACAGCCT	TTTATGTTT	GGATGGACAA
	1001	AAAGATCTC	TACTTTGTTG	AAATGGTGG	GATGCGGAC	AAATGTCAGA
	1051	AGGGTACATC	TGTGTGAAAG	CTGGACGAA	CCCCAAC	GGTTACACGA
	1101	GCTCTGACAC	ATTAGCTGG	GCCTCTTAT	CGCTGTTTCC	ACTCATGACT
	1151	CAAGACTCT	GGGAAACCTT	TTTACAGTTG	ACATTAC	CAGCTGGAA
35	1201	AACCTACATG	ATCTTTCTG	TCCCTGGTAA	TTCTTGGC	TCATTTATT
	1251	TGGTGAAC	TGATCTGGCT	TTGTTGGCC	TGGCTTATGA	GGAAACAAAT
	1301	CAGGCCACAC	TGGAGGAGGC	TGAGCAGAA	GAGGGAGGT	TTCAGCAGAT
	1351	GTTGGAGCAG	TTGAAAAGAC	AGCAAGAGGA	GGCTCAGGCG	GTGGCAGCTG
	1401	CCTCAGCAGC	GTCCAGAGAC	TTCACTGGGA	TAGGAGGGTT	AGGAGAACTT
40	1451	CTGGAGATT	CTTCAGAACG	TTCAAGTTG	AGCTCCAAGA	GTGCCAAGGA
	1501	GTGGAGGAAT	CGGAGGAAGA	AGAGGAGACA	GAGGGAGCAC	TTGGAGGGAA
	1551	ACACACAGC	CGAAGGAGAC	AGGTTTCCC	AGTCGGAAATC	AGAACGACAGC
	1601	GTCAAAGCGA	GGAGTTTCT	GTTCTCCCTG	GATGGGAACC	CGCTGAGGG
	1651	CGACAAGAAG	CTGTGCTCT	CCCATCAGTC	TCTCTGAGT	ATCCGGGCT
45	1701	CCCTGTTTC	CCCAAGAGCC	AAATGCAAA	CGAGCATTTT	CAGCTTCAAGA
	1751	GGTGGGGAGA	AGGAGCTGG	GTCTGAGAT	GACTTTGGG	ATGATGAAACA
	1801	CAGCACCTT	GAAGATAGCG	AGAGCAGGAG	AGACTCACTG	TTTGTGGCC
	1851	ACAGACCTGG	AGACCGAGGC	AAAGTAAACG	TTAGTCAGGC	CAGTATGTC
	1901	TCCAGGATGG	TGCCAGGGCT	TCCAGCCAAT	GGGAAGATGC	ACAGCACTGT

1951 GGATTGCAAT GGTGTTGTTT CCTTGGGTAC CACCACTGAA ACAGAAAGTC  
 2001 GGAAGAGAAG CGTAACTCTT TACAGATCTT CGATGAAAT CGTGGGAGAT  
 2051 TCCCTGGGAA GACAAGAGC CATGAGCATC GCCAGTATCC TGACCAACAC  
 2101 GATGGGGAA CTTGAAGAAT CTAGACAGAA GTGTCCACCA TGCTGGTATA  
 5 2151 GATTGGCAA TGTGTTTTG ATCTGGACT GTTGTAATTG ATGGTGAAA  
 2201 GTAAAGCATC TTGTAATTG GATTGTGAT GATCCATTG TTGACCTGCG  
 2251 CATCACCATC TGCATCGTGT TAAACACACT GTTCATGCC ATGGAGACT  
 2301 ACCCGATGAC GGAGCAAGTC AGCACTGTG TGACCGTGGG AAACCTGGTC  
 2351 TTCACGGGA TCTTCACAGC CGAGATGGTC TGAAAATCA TCGCAATGGA  
 10 2401 TCCCATTAC TATTCACAG AGGGCTGGA TATCTTGTAT GGAATTATG  
 2451 TTAGCTGAG TTTAATGGAG CTTGGCTGG CAAACGTGGA GGGGCTGTC  
 2501 GTGCTTCCGT CTTTCAGACT GCTGGCACTG TTCAAGTTGG CAAATCTG  
 2551 GCCCACACTG AATATGCTCA TTAAGATCAT CGCAACTCG GTGGGCCAC  
 2601 TGGGACACTT GACCCCTGGT CTGGCCATCA TCAGTCTTAT TTTTGCCTG  
 15 2651 GTCGGCATGC AGCTGTTGG AAAGAGCTAC AAGGAGTGTG TTGCAAGAT  
 2701 CAATGAGGG TGCAAGCTCC CGCGCTGGCA CATGACGAC TTCTTCACT  
 2751 CTTTCCTGAT AGTGTGTTGG GTGCTGTG TGGGAGTGGAT AGAGACCATG  
 2801 TGGGACTCTA TGGAGCTCC CGGGCAGAC ATGTCCTTA TTGTTTAT  
 2851 GTTGGCTATG GTGATTGGGA ACCTGTGGT TCTGAACCTC TTCTGGCCT  
 20 2901 TATTGTTGAG TTCTCTTATG TCAGACAAAC TTGCTGTAC GGACGATGAT  
 2951 AACGAAATGA ACAACCTCCA GATCGGGTGG GGAAGGATGCA AAAAGGGAT  
 3001 TGATTATGTG AAAAATAAGA TACCGGAGTG CTTCGGAAA GCGTTTTTA  
 3051 GAAAGCCGA AGTGTAGAA ATCCACGAGG GGAACAAAT AGACAGCTGC  
 3101 ATGTCCTAA ACACGGGGT AGTTGAATA AGCAAGAGC TTAACTACCT  
 25 3151 TAAAGACGGT AACGGAAACCA CCAGTGGGT GGGTACTGGA AGCACTGG  
 3201 AGAAATACGT AATTGATGAA AATGACTACA TGTCATTATC CAACAAACCC  
 3251 AGCCTCACCG TGACGGTGGC ATTTGCGTGG GGAGAGTCTG ACTTTGAAA  
 3301 TTAAACACG GAAGAGTTTA GCAGTGTGAG AGAACCTGGA GAAACCAAGG  
 3351 AGAAATTTAA TGCAACCCAGC TCTCTGGAAG GAAGCACAGT TGACCTGTG  
 30 3401 CGGCCCGAG AAGGTGAAAC AGCTGAATT GAACCTGAGG AGGACCTTAA  
 3451 GCGAACAGCT TCCTTACTG AAGGTGAT TAAAAAATT CCCTCTGCC  
 3501 AAGTAAGTAC GGAAGAAGGT AAAGGAAAAA TCTGTTGGA TCTTAGGAA  
 3551 ACCTGTATA GCATTGTGGA ACACAACTGG TTGAGACAGT TCATTGTT  
 3601 CATGATTCTC CTCACTGTG GTGCTTGTG CTTGAAGAT ATATACTATG  
 35 3651 AGCAACGGA GACCATCAA ACCATGCTGG AGTATGCTGA CAAAGCTTC  
 3701 ACTTACACTT TCATCCTGGA AATGCTCTC AAATGGGTGG CCTATGGATT  
 3751 TCAACACTT TTCAACCATG CCTGGCTGG CTGGACTCTC TTGATTGTT  
 3801 ATGTTCTTT GTGTTAGCTG GTGGCCAAAG CTCTGGCTA TTCAAGACTT  
 3851 GGTGCAATCA AATCCCTACG TGAGGAGGA GCTCTGAGGC CGCTCCGAGC  
 40 3901 CTTATCCCG TTTGAAGGCC TGAGGGTGT TGTGAACCTC TTGTTGTTG  
 3951 CAATCCCCTC CATCATGAAT GTGCTACTGG TGTCCTCAT TTCTGGTTA  
 4001 ATCTTCTGTA TCATGGGTGT GAATCTGTT GCTGGAAAGT TCTATCACTG  
 4051 TGTTAACACTG ACAACGGGCA GCATGTTGCA CATGAGTGA GTCACAACTT  
 4101 TCAGGGACTG TCAGGCTCTT GGCAAGCAAG CCCGATGGAA GAATGTGAAA  
 45 4151 GTCAACTTG ACAAATGTTG GGCTGGCTAC CTGGCATGTC TGCAAGTGGC  
 4201 CACATTCAA GGCTGGATGG ATATTATGTA TGCAAGCTGTG ATTCTACCGG  
 4251 ACGTCAAACG GCAGCCCTGA TATGAAGAAA ATCTGTACAT GTATCTGTAC  
 4301 TTGTCATCT TCATCATCTT TGGGTCGTC TTCACTCTAA ATCTTATTCAT  
 4351 CGGCGTCTCA ATAGACAACT TCAACCCAGA GAAGAAGAAG TTGGAGGTC  
 50 4401 AAGACATCTT TATGACAGAA GAGCAGAAAA AGTACTACAA TGCAATGAAG  
 4451 AAACCTGGT CCTAAAAAAC TCAGAAAGCC ATCCCTCGAC CTGCAAACAA  
 4501 ATTTCAAGGA ATGGTCTTGT ACTTTGTAC CAGACAATG TGTTGACATCA  
 4551 GCATCATGAT CTCATCTGCTC TCAACATGG TGACCAATGAT GGTGGAAACG  
 55 4601 GACGACCCAGA GCAAAATCAT GACCCCTGGT TTGTCGGAA TCAACCTGGT  
 4651 ATTCACTGTC CTCTTCAGTG GGGAGTTCT GCTGAAGCTC ATCTCTCTCA  
 4701 GATACTACTA CTTCACGATT GGCTGGAACA TCTTGTACTT TGTTGGTGTG

4751	ATTCTCTCAA	TTGTAGGAAT	GTTCCCTTGCT	GAGCTGATAG	AGAAGTATT	
4801	TGTGTCCTCT	ACCCCTTCC	GAGTCATCGG	CCTGGCCAGG	ATTGGCAGAA	
4851	TCTTACGGC	GATCAAAGGC	GCAAGGGGA	TCCGACCGT	GCTCTTGTCT	
4901	CTGATGATGT	CCCTTCTCGC	GCTGTTCAAC	ATCGCCCTCC	TGCTTTCTCT	
5	CGTGTGTTG	ATCTACGCCA	TCTTGGGAT	GTCCAACTTT	GCCTATGTTA	
5001	AAAAAGAGGC	TGGAATTGAT	GACATGTTCA	ACTTTGAGAC	TTTGCGCAAC	
5051	AGCATGATCT	GCCTGTTCCA	AATCACACC	TCTGGGGCT	GGGATGGACT	
5101	GTTGGCCCCC	ATCCCTCAACA	GTGACACTCC	TGACTGTGAC	CCTGATGCAA	
5151	TTCACCTCGG	AAAGCTCAGT	AAGGGAGACT	GTGGGAACCC	ATCTGTGGGG	
10	5201	ATTTCCTTT	TTGTCAAGCTA	CATCATCATA	TCCTTCTGG	TTGTGTTGAA
5251	CATGTACATT	GCTGTCATCC	TGGAGAACCT	CAGCGTTGCC	ACAGAAAGAA	
5301	GTGAGAGGCC	CCTGAGTGG	GACGACTT	AGATGTTCTA	CGAGGCTCGG	
5351	GAGAAGTTGC	ACCCCTGAGC	CACCCAGGT	ATAGAGTTCT	GCAAGCTCTC	
5401	TGACTTGTCA	GCTGCCCTGG	ATCCTCCCTT	CCTCATGCCA	AAGCCAAACA	
15	5451	AAGTCCAGCT	CATTGCCATG	GACCTGGCCA	TGGTGAAGTGG	AGACCGCATC
5501	CACTGCCTCG	ACATCTTATT	TGCTTTTAA	AAGCGGGTCC	TGGGTGAGAG	
5551	TGGAGAGATG	GATGCCCTTC	GAATCCAGAT	GGAAGATCGG	TTCATGGCTT	
5601	CCAATCCCTC	CAAGGCTCT	TATGAGGCCA	TTACACCCAC	TCTGAAGGGC	
5651	AAACAAGAGG	AGGTGCTTC	TGCTATCATT	CAGCGTAATT	ACAGATGTTA	
20	5701	TCTTTTAAAG	CAAAGGTTAA	AAAACATATC	AAATACGTT	GACAAAGAGA
5751	CAATCAAGG	GAGGATTGTC	TTGCTTATAA	AAGGGAGAT	GGTTATTGAC	
5801	AAATTAATG	GGAATCCAC	CCCGAAAAG	ACAGATGGGA	GTTCTCTAC	
5851	CACCTCCCTT	CCTTCTATG	ACAGTGTAA	AAAACCAGAT	AAGGAAAAGT	
5901	TTGAGAAAAGA	CAAACCAGAA	AAAGAAAGCA	AAGGGAAAGA	GGTCTGAGAG	
25	5951	AATCAAAAGT	AAAAAAACAA	AAACAAAAAA	ATTCAAAAAA	TTTAAAGGAA
6001	AAACAAAGAA	ATGTCCTTGT	AATCAATTGT	TTACAGCCTC	TGAAGGTA	
6051	GTGTCGGTGT	CAACTGGACT	C			

30

Table 2. Amino acid sequence of mNa<sub>1.3</sub>  $\alpha$  subunit (SEQ ID NO:2)

35	MAQALLVPPGPESPRFLFTRESLAAIEKRAAEEKAKKKPEQDIDDENPK
	PNSDLEAGKNLPLPIYGDIPPEMVSPEPLEDPIYVSKKTFVVLNKGAIF
	RFSATSALEYLTLPLNPRVKIAKILVLHSLFSMLIMCTILTCNCVFMTLSNP
	PDWTKNVEYFTPTGUYTFESLKLILARGFCLEDFPTFLRDPWNWLDFSVIVM
	AYTVEFVDLGNVSALRTRFLVRLTIVSPGLKTTIVGALIQSVVKLSDLV
	MILTFVCLSVFALIQLQLFMGNLNRKNCQWPPPSDSAFEINTTSYFNGTMD
40	SGNTFVNVTMSTFWKDIADDSHFYVLDGQKDPLLCGNGSDAGQCPEGY
	ICVKAGRNPVNGYTSFDTFSWALFLSLFLRFLMTQDWNLYQLTLRAAGKTY
	MIFFLVLFGLGSYFLVNLNLLAVAMAYEEQNQATLEEEAEQKEAEFQOMLE
	QLKKQQEEAQAAVAAASASRDFSGIIGGLGELLESSSEASKLSSSAKEWWR
	NRRKRRQREHLEGNRHPEGDRFPKSESEDSVKRSFLFSLDGNPLSGDK
45	KLCSPHQSLLSIRGSLFSRPRNSKTSIIFSFRGRAKDVGSENDFADDEHST
	FEDSESRDLSLFVPHRPGERRNSNVSQASMSSRMVPGPANGKMHSTVDC
	NGVVSGLTTTETEVRKRKRLSSYQISMEMLEDSSGRQRAMSIASILINTME
	ELEESRQKCPCCWYRFANVFLIWDCCDSWLKVKHVLNLIWMDPFVLDLAIT
	ICIVLNTLPFAMEMHYPMTEOFQSSVLTVFVGNLVFTGIFTAEMVLKIIAMDPY
50	YYFQEGWNIFDGIIVSLSLMEGLANVEGLSVLRSFRLRUVFKLAKSWPT
	LNLLNLIKIGNGSVGLGNLTVLALIIVFIFAVVGMQLFGKSYKECVKCLNE
	DCKLPLRWHMNDFPHSFLIVFVRLCGEWIETMNDCMEVAGQTMCILIVPMLV
	MVIGNLVNLFLALLSSFSNDDLAATDDNEMNNLQIAVGRMOKGIDY
	VVKIKIRECFRKAFFRKPKVIEIHEGNKIDSCMSNNNTGVEIASKELNYLK
55	GNGTTSGVGTGSSEVEKYVIDENDYMSFINNPSLTVTVPIAVGESDFENLN
	TEEFSSSELEESKEKLNATSSSEGSTDVDAAPPRGEQAEIEPEEDLKPE

ACFTEGC1KKFPFCQVSTEEGKGK1WWNLRKTCYS1VEHNWFETF1VFMI  
 LLSGGALAFED1YIEQRKTIKTMLEYADKVFYTIFILEMLLKVNAYGFOT  
 YFTNAWCWLDFLIVDLSVLSVANALGYSELGAIKSRLTRALRPLRALS  
 RFEGRMRVVVNAVGAIIPSIMNVLLVCLIFWLI1S1MGVNLFAGKFYHCVN  
 5 MTTGSMFDMSEVNNFSDCQALGKQARWNKVNFDNVGAGYLALLQVATF  
 KGWMIDMYA1VDSRDVKLQPVYEENLYM1YFV1F1FGSFT1NLFIGV  
 10 IIDNFNQOKKKFFGQD1FMT1EQQKKY1NAMEK1LGSKKPQK1PRPANKFQ  
 GMVFDFTVROFDISIM1LICLNMTMMVETDDQSKYMTLVL1SR1NLVFI  
 VLF1TGEFLKL1L1R1Y1Y1FT1G1W1N1F1D1V1V1L1S1V1GMFLAEL1EKYFVS  
 PTLFRVIRLAR1G1RL1K1G1K1G1R1T1L1F1M1S1P1A1F1N1G1L1F1V1M  
 15 FIY1A1FGMSNF1Y1V1K1E1G1D1D1M1F1E1F1P1G1S1M1C1L1F1Q1T1S1A1G1W1D1L1A  
 PI1NS1A1P1D1C1P1D1P1A1P1N1S1E1S1D1E1F1M1F1Y1E1W1K1F1D1P1A1T1S1  
 20 PI1V1E1N1F1S1V1T1E1S1A1E1P1S1E1D1F1E1M1F1Y1E1W1K1F1D1P1A1T1S1  
 AA1LD1P1L1IA1K1P1N1K1V1Q1I1A1M1D1L1P1M1V1S1G1D1R1H1C1L1D1L1F1A1P1T1K1R1V1G1E1S1G1E1  
 25 MD1AL1D1P1L1I1A1K1P1N1K1V1Q1I1A1M1D1L1P1M1V1S1G1D1R1H1C1L1D1L1F1A1P1T1K1R1V1G1E1S1G1E1  
 K1Q1R1K1N1S1N1Y1D1K1T1K1G1R1V1L1P1K1G1D1M1D1K1L1N1G1N1S1T1P1E1K1T1D1G1S1S1T1T1  
 P1P1S1Y1D1S1V1T1K1P1D1K1F1K1F1K1P1K1P1E1K1S1G1K1  
 30 P1P1S1Y1D1S1V1T1K1P1D1K1F1K1F1K1P1K1P1E1K1S1G1K1  
 SEQ ID NO:1

All references cited herein, whether in print, electronic, computer readable storage media or other form, are expressly incorporated by reference in their entirety, including but not limited to, abstracts, articles, journals, publications, texts, treatises, internet web sites, databases, software packages, patents, and patent publications. A number of embodiments of the invention have been described. Nevertheless, it will be understood that various modifications may be made without departing from the spirit and scope of the invention. Accordingly, other embodiments are within the scope of the following claims.

SEQ ID NO:1

ATGGCCCAGGCACTGCTGGTACCCCGGGACCTGAGAGCTTCC  
 GCCTTTCACTCGAGAATCTCTGCTGCTATCGAAAGCGTCTGCAGAA  
 GAGAAAGCCAAAGAAACCCAAAGAGAACAGACATGAGATGAGAACAA  
 35 GCGAAAGCCAAACAGTGACTTGGAAAGCTGGGAAGAACCTTCCATTATCT  
 ATGGAGACATTCTCCAGAGATGTTGCGAGCTCTGGAGGACCTGGAC  
 CCTCTACTAGCTGACTAAAGAACATTCTTGATGTTGAAATAAAGGGAAAGGC  
 AATTTCGATTCTGAGCTCCGCTCTGTATATTAACTTCACTAA  
 40 ACCCGTTAGAAAATTGCTATTAGATTGGTACACTCTTATTCTAGC  
 ATGCTTATCATGTTGACTATTGAGCCAACCTGTTGATTTATGACATTGAG  
 CAATCTCTTGACTGGACGAAGATGTTAGAGTACACATTCACTGGATCT  
 ATACCTTGTAGTCACTTAAAGATCTGGCCAGAGATTCTGCTTAA  
 GATTTCACATTCTCTGTCAGCCATGGAAACTGGCTGGATTTCAGTGTCT  
 45 CGTGTGCGATATGTGACAGAGTTGTGAGCTGGCAATGTCTCAGCGC  
 TGAGAACGTTGAGTTCTCGAGCATTTGAAAACAATATCAGTCATTCCA  
 GTGTTAAAGACCATCTGGGGGCTGATCCAGTCAGCTGGTGAAGAACGCTGTC  
 TGACGTCATGATCACTACTGTTCTGCTCTGAGCTCTTGTCTCATCG  
 GGCTGCACTCTCATGGCAACCTGAGGAATAATGCTGAGCTGGCCT  
 CCAACCGATTCTGTTGAGATCAACACTACTTCCTACTTCAATGGCAC  
 AATGGACTCAAATGGGACATTGTTAATGTAACAAATGAGCACATTCAACT

GGAAGGACTATATCGCAGATGACGCCACTTTATGTTTGGATGGACAA  
 AAAGATCCTTTACTTIGGGAAATGGTCCGATGCGAGGAAATGTCAGA  
 AGGGTACATCTGTGTAAGGGCTGGGAAACCCCAACTACGGTTACAGA  
 GCTTTGACACATTAGCTGGGCTTCTTATCGTGTTCGACTCATGACT  
 5 CAAGACTACTGGGAGAACCTTACCGATGACATTACGTGCACTGGGA  
 AACCTACATGATCTTTCTGCTCTGGTAAATTCTGGCTCATTTTATT  
 TGGTGAACCTTGATCCTGGCTGTGGTGGCCTATGGCTATGAGGAACAAAAT  
 CAGGGCCACTGAGGAGGCTGAGCAGAAAGGGGGAGGTTTCAGCAGAT  
 GTTGGAGCAGTTGAAAAGCAGAAGGAGGGCTCAGGGGGCAGCTGG  
 10 CCTCAGCAGCGTCCAGAGACTTCAGTGGAATGGAGGGTTAGGAGAACCT  
 CTGGAGAGTTCTCAGAGCTTCAAGTTGAGCTCCAAAGTGGCAAGAG  
 GTTGGAGAATCGGAGGAAGAGGAGACAGGGAGCACTGGAGGGAA  
 ACCACAGACCGAAGGGAGCAGGTTTCCAACTGCGAATCAGAACAGC  
 GTCAAGCGAAGGGAGTTCTGGTCTCCCTGGATGGGAAACCCCTGAGCGG  
 15 CGAGAAGAAGCTGTCCTCCCATCAGTCTCTTGAGTATCCGTGGCT  
 CCGTGTTCCTCCAAAGCAGCAATAGCAAACAGAGCATTTCAGCTTCAGA  
 GTTCGGCGGAAGGGAGCTGGGTTCTGAGAAATGACITTGCGGATGATGAA  
 CAGCAGCTTGAAGATAGCAGAGCAGGAGGAGACTCACTGTTGCCCC  
 ACAGACTGGAGGCAGCAGAACAGTAACGTTAGTCAGGCAGTATGTC  
 20 TCCAGGATGGTCCAGGGCTCCAGCCAATGGGAAGATGCAAGCACTGT  
 GGATGCAATGGTGTGGTTCTCTGGTACCCACTGAAACAGAAGTC  
 GGAAGAGAAGGCTAAGTCTTACAGATCTGATGGAAATGCTGGAGGAT  
 TCCCTGGAGACAAAAGGCCATGAGCATAGCCAGTATCTGACCAACAC  
 GATGGAGGAACCTTGAAGATAGCAGAAGTGTCCACCATGCTGGTATA  
 25 GATTGCCATGTGTTTTGATCTGGACTTTGTGATTCACTGGTGGAA  
 GTAAAGCATCTGTGTAATTGATGTTGATGGATCATTGTTGACCTGGC  
 CATCACCATCTGATCGTGTAAACACACTGTTCATGGCATGGAGCACT  
 ACCCGATGCGAGGAGCACTGGCAGTGTGCTGACGGTGGGAAACCTGGT  
 TTACCGGGATCTTCAACCGCGAGATGTTCTGAAAATCATGCCAATGG  
 30 TCCCTATTACTATTCCAGAGGGCTGGAAATATCTTGTGAGGAAATTATG  
 TTAGCTGAGTTAACTGGAGCTTGGCCTGGAAACCTGGAGGGCTGTCC  
 GTGCTCTGGTCTTCAGACTGCTGCGAGCTTCAAGATGGCAAATCTG  
 GCCCACACTGAATATGCTCATGGAAACTCGGCAACTCGGTGGGCGCAC  
 TGGGAAACCTGACCCTGGTCTGGCCATCATGCTCTTATTGGCGTG  
 35 GTGGCATGCCAGCTGGTGGAAAGAGCTAACAGGAGTGTGTTGCAAGAT  
 CAAATGAGGACTGCAAGCTCCGGCGCTGGCACATGAAACGACTTCTCACT  
 CCTTCTGATAGTCTGGCTGTGGGAGTGGATAGAACCATG  
 TGGGACTGATGGAGGTCGGGGCCAGAGCATGTGCTTATGTTGTTAT  
 GTTGGTATGGTGTGGTGGAAACCTGGTGGTTCTGAAACCTTCTGGCCT  
 40 TATTGTTGAGTTCTTCTGGTCAAGAACCTTGTCTGGCTACGGAGCATGAT  
 AACGAAATGAACACCTCCAGATCGCGGTGGGAAGGATGCAAAGGGGAT  
 TGATTGAAAATTAAGGAGCTGGGAGTGGTCCGAAAAGGTTTTTA  
 GAAAGCGGAAAGTGTAGAAAATCCAGAAGGGAAACAAATAGACAGTC  
 ATGTCCTAAACACGGCGTAGTGGAAATAGCAAAGAGCTTAACACT  
 45 TAAAGCAGGTAACGGAACCCAGCTGGCGTGGGTACTGGAGAGCATGTGG  
 AGAAAATACGTAAATGTGAAATGACTACATGTCATTGATCACAAACCCC  
 AGCCTCACCGTACGGTGGCAATTGGCGTGGAGAGTCTGACTTTGAAA  
 TTAAACACGGGAAGGTTAGCAGTGAATGAGAATGGAAGGAAAGCAAGG  
 AGAAAATTAATGCAACCCAGCTTCTGGAAAGGAGCACAGTGTGCT  
 50 CGGCCCCGAGAAGGTGAACAGCTGAAATTGAAACCTGAGGAGGACCTTAA  
 GCCAGAGCTTGTCTTACTGAGGATGCAATTAAAAAATTCCTTCTGCG  
 AAGTAAGTACGGAGAGGTTAAAGGAAAATCTGGTGGAACTTCTAGGAAG  
 ACCTGCTATAGCATGGACACAACCTGGTTGAGACGCTTATTGTT  
 CATGATTCTCTCAGTAGTGGTGTCTTGGCTTGTGAGGATATACATTG  
 ACCAACGGAGAACGACATCAAACCATGCTGGAGTATGCTGACAAAGTC  
 ACTTACATCTTCATCCCTGAAATGCTCTCAAAATGGGTGGCCTATGGATT  
 TCAAAACCTTACCAATGCCCTGGTGTGGACTTCTGATTGTT

ATGTTCTTGGTTAGCTGGGCCAACGCTTGGCTATTCAGAACCT  
 GGTGCCCCATCAAATCCCTACCGGACCTGAGAGCTCTGAGGCCCCCTCCGGAGC  
 CTTATCCCGCTTGAAGGCATGAGGGTGTGTGAACGCTCTGTGGTG  
 CAATCCCCCTCCATCATGAATGTGACTGGTGTGCCATCTCTGGTTA  
 5 ATCTTAACTATGGGGTGTGAATCTGTTGCTGGAAAGTCTATCACTG  
 TGTAAACATCACACAGGGCAGCATGTCGACATGAAGTGAAGTCACAAATT  
 TCAGCGACTGTGAGGCTTGGCAAGCAAGGCCGATGGAAAGAATGTGAAA  
 GTCACTTTGACAATGGGGCTGGGCTACCTGGCATGGCTCAAGTGGC  
 CACATTCAAAGGCTGGATGGATATTATGTATGCAGCTGGGATTACAGGG  
 10 ACGTCAAACGTCAGGCCATGATAGAAAGAAATCTGTACATGATCTGTAC  
 TTGTCATCTCATCTTGGGCTGGTCTTCACTCTAAATCTATTCTAT  
 CGCGCTCATGACAACTTCAACCCAGAGAAAGAAGTTGGAGGTC  
 AAGACATCTTATGACAGAAAGAGCAGAAAAAGTACTACAATGCAATGAAG  
 AAACCTGGCTCCAAAAAAACCTCAGAACGCCATCCCTGACCTGCAAAACAA  
 15 ATTTCAAGGAATGGCTTGTACTGTAAACCAGACAAGTGTGACATCA  
 GCATCATGATCTCATGCTCAACATGGTGACCATGATGTGGAAACCG  
 GACGACAGAGAACATGACCCCTGGTTTGTCCGAATCAACCTGGT  
 ATTATCCTGCTTCACTGGGGATTCTGTGAAGCTCATCTCTCTGA  
 GATATACTACTACTCAGGATGGCTGGACATCTTGTACTTTGTGGTGGTG  
 20 ATTCTCTCAATTGTAGGAATGTTCTTGTCTGAGGCTGATAGAGAAGTATT  
 TGTGTCCTACCCCTTCCGAGTCATCCGGCTGGCCAGGATTGGACGAA  
 TCTCTACGGCTGATCAAAGGCCAAGGGGATCCGACCTGCTTGTGCT  
 CTGATGATGTCCCTTCTGGCTGTTCTGTGAAGCTCATCTCTCTGA  
 CGTCTGTTCATACCGCATCTTGGGATGTCACATTGGCTATGTTA  
 25 AAAAGAGGCTGGAAATTGATGACATGTTCAACTTGGACTCTTGGCAAC  
 AGCATGATCTGCCGTGTCACCAATCACCCACTCTGGGGCTGGGATGGACT  
 GTTGGCCCTACCTCAACAGTCGACCTCTGACTGTGACCTGTGATGCAA  
 TTCACCTGGAAAGCTAGTGAAAGGAGACTGTGGAAACCCATCTGTGGGG  
 ATTTTTCTTTTGTCAGCTACATCATCATATCTCTCTGGTGTGTGGTGA  
 30 CATGTACATCTGCTGTCATCTGGGAAACTCTAGCGTGGCCACAGAAAGAA  
 GTGCGAGGCCCTGAGTGAGGAGCAGTTGAGATGTTCTACCGAGGCTGG  
 GAGAAGTTGACCCCTGACGCCACCCAGTTCATAGAGTTCTGCAAGCTCTC  
 TGACTTTGAGCTGCCCTGGATCCTCCCTCTCATCGCAAAAGCCAAACA  
 AAGTCCAGCTATTGGCATGGACCTGGCCATGGTAGTGGAGACCCGATC  
 35 CACTGGCTGGACATCTTATTGCTTACAAAGCGGGCTCTGGTGAGAG  
 TGGAGAGATGGATGCCCTCGAACATGGAGATCGGTTCTAGGGCTT  
 CCAATCCCTCCAGGCTCTCTATGAGGCCATTACCAACACTCTGAAGGCC  
 AAACAAGAGGGAGGTGCTGCTGCTATCATTAGCTTAATTACAGATGTTA  
 TCTTTAAAGCAAGGTTAAAAACATCAAATACAGTATGACAAGAGA  
 40 CAATCAAGGGAGGAGATTGTCCTGGCTATAAAAGGAGATATGTTTATTGAC  
 AAATTAAAGGAAATTCCACCCAGAAAAGACAGATGGGAGTTCCCTCAC  
 CACCTCCCTCTCTATGACAGTGTAAACAAACAGATAAGGAAAAGT  
 TTGAGAAAGACAACCAGAAAAAGAAGCAAGGAAAGGAGGTCTGA

45 SEQ ID NO:2

MAQALLVPPGPESFRLFTRESLAAIEKRAAEEKAKPKKEQDIDDENKPK  
 PNSDLEAGKNLPIYGDIPPEMVSPEPLEDLPYVYSKKTFVVLNKGKAIF  
 RFSATSLAUYILTPNPNVKIAIKILVHSLFSMLIMCTILTNCVFMTLSNP  
 50 PDWTKNVEYFTGIYTFESLKIILARGPCLEDFTFLRDPWNWLDFSVIVM  
 AYVTEFVDLGNVSALRFTVRVLRAKTISVPLGLTKIVGALIQSVKQLSDV  
 MILTFVFLCSVFA1GLQLFMGNLNRKCLQWPBSDSAFEINTTSYFNGTMD  
 SNGTFVNVTMSTFNWKDYIADDSHFYVLDGQKDPLLCNGNSDAGQCPGEY  
 ICVKAGRNPNYGTSFDTFSWAFLSLRFLMTQDYWENLYQLTRLRAAGKTY  
 55 MIFPVLVIFLGSFYVLNL1AVAVAMAYEEQNQATLLEAEQKEAEFQOMLE  
 QLKKQQEEAQAVAAAASASRDFSGIGGLGELLESSSEASKLSSKSAKEWR  
 NRRKKRQRREHLEGNHRPEGDRFPKSESEDVKRRSFLSLDGNPLSGDK

KLCSPHQSSLISRGLSFLSPRRNSKTSIFSFRGRAKDVGSENDFADDEHST  
 FEDSESRDRSFLVFPHRPGERRSNVSQASMSSRMVPGLPANGKMHSTVDC  
 NGVVSLSGTTTETEVRKRLSSYQISMEMLEDSSGRQRAMSIASILNTMEE  
 ELEESRQKCPWCYRFANVFLIWDCDSWLKVKHVLNLIVMDPFDLAIT  
 ICIVLNLTFMAMEHYPMTEQFSSVLTVGNLVFTGIFTAEMVLKIIAMDPY  
 YYFQEGWNIFDGIIVSLSLMLGJANVBLGSVLRSFRLRVFKLAKSWPT  
 LNMLIKIIGNSVAGLNLTVLALIIVFIFAVVGMQFLGKSYKECVCKINE  
 DCKLVRWHMDPFLHSFLIVFVRVLCGEWIETMMDCMEVAGOTMCLIVPMLV  
 MVIGNLVVNLFLAILLSSFSDDNLAATDDNEMNNLQIAVGRMOKGIDY  
 VKNKIRECFRKAFFRKPKVIEHEGNKIDSMSNNNTGVEEISKEMLNLYKD  
 GNGTTTSLFVCGTGSVEVKVIDENPSSFLINNPSLTTVTPIAVGESDFENLN  
 TEFFSSESELEBESKEKLNTSSSEGSTVDVAPPREGEQAEIEPEEDLKPE  
 ACPTEGCIKKPFPCQVSTEEGKGKIIWNLRLKTCYISVEHNNWFETIVFMI  
 LLSGGALAFEDIYEQLRKTIKTMLEYADKVFYIIFILEMLLKWNVAYGQQT  
 10 YPTNAWCWLDFLIVDVLVSLSLMLGJANVBLGSVLRSFRLRVFKLAKSWPT  
 RCEGMRRVVAVALNVAIAPSIMNVLVLCLIFWLIFSIMCVNLFAKGKFYCVN  
 MTTGSMFDMSVNNFSDCQALGKQARWKNVKVNFDNVGAGYALLQVATF  
 KGWMIDMYAADSRSVQLQPVYEENLYMLYFVIFIIFGSFFTINLFIGV  
 15 IDNFNQOKKKFFGQDIFMTTEQQKKYNAMEKMLGSKPKQPIPRPANKFQ  
 GMVFDFTVQFDISIMLICLNMTMMVETDDQSKYMTLVLRSRINLVFI  
 VLFPTGEFLKLISLRRYYFTGWNIFDFVVVILSIVGMLFALIEKYFVS  
 PTLFLFRVIRLARIGRLRIKGAKGIRTLFLALMSPALPNIGLLFLVM  
 FIYIAFGMSNFAYVKKKEAGIDDMNFETPGNSMCLFQIITSAGWDGLLA  
 20 PILNSAPPDCPDAIHPGSSVKGDCGPNPSVGIFFFSYIIISFLVUVNMY  
 IAVILENFSVATEESEAELPSLEDFDEMFEVWEEKFDPPDATQFIEFCKLSDF  
 AAALDPPLIAKPNVQLIAMDLPMVSGDRHICLIDLFAFTKRVLGESGE  
 MDALR1QMEDRPMASNFVSYEPITTLKRKQEEVSAAIIQRNYRCYLL  
 KQLRKNISNTYDKEITKGRIVLPKGMDV1DKLNGNSTPEKTDGSSSTTS  
 25 PPSYDSTSVPKDPKEKEFDKPEKESKGKEV  
 30

## SEQ ID NO:3

35 TGAAAAGATGGCCCAAGGCACTGCTGGTACCCCGGGACCTGAGAGCTTCC  
 GCTTCTTCACTCGAGAATCTCTCTGCTATCGAAAAGCGTCCTGCAGAA  
 GAGAAAGCCAAGAAAACCCAAGAAAACAAGACATITGACGATGAGAACAA  
 GCCAAGGCCAACAGTGACTIONTGGAGCTGGGAAGAACCTTCATTTATCT  
 ATGGAGACATTCTCCAGAGATGGTGTGGAGCCCTCTGGAGGACCTGGAC  
 40 CCCCTACTAGTCAGTAAAGAAAACCTTTGTAGTTGAATAAAGGGAAAGGC  
 AATTTCGATTCACTGGCACCTCCGCTTGTATATTTAACCTTCACTAA  
 ACCCGTTAGGAAAATTGGTATTAAAGGTATTGGTACACTCTTTACGC  
 ATGCTTATCATGTGCACTTTGACCAACTGTGATTATGACATTGAG  
 CAATCCTCTGACTGGACAGAACATGTAGAGTACACATTCACTGGATCT  
 45 ATACCTTTAGTCACTTAAAGATCTTGGAGGACAGGATTCTGGCTTAAAGA  
 GATTTCACATTCTCGTGGACCATGGAACTGGCTGGATTCTAGTGTCT  
 CGTGATGGCATATGTGACAGAGTTGTGGACCTGGCAATGCTCAGCGC  
 TGAGAACGTTCACTGGGACATTGGACATTGAAACAAATATCAGTCATTCCA  
 GGTAAAGACCATCTGGGGCCCTGATCCAGTCGGTGAAAGAGCTGTC  
 50 TGACGTCATGATACTCACTGTGTTCTGCTTGAGCGCTTTCGCTCTCATCG  
 GGCTCGAGCTCTCATGGCAACTGGAAATAATGCTTGCAGTGGCCT  
 CCAAGCGGATCTGCTTGGAGATCAACACTACTTCACTTCAATGGCAC  
 AATGGACTCAAATGGGACATTGTGTAATGTAACAAATGAGCACATTCAACT  
 GGAAGGACTATATCGCAGATGACGCCACTTTATGTTTGGATGGACAA  
 55 AAAGATCCTTACTTGTGGAAATGGTCCGATGCAAGGACAAATGTCAGA  
 AGGGTACATCTGTGTAAGGCTGGACGAAACCCCAACTACGGTTACACAG  
 GCTTGACACATTAGCTGGGCTTCTTATCGCTGTTTCGACTCATGACT

CAAGACTACTGGGAGAACCTTACAGTTGACATTACGTGCAGCTGGAA  
 AACCTACATGATCTTTCCTGCTCTGGTAAATTCTTCTGGGCTCATTTTATT  
 TGGTGAACCTTGATCCTGCGCTGGTGGCCATGCCATAGGGAAACAAAT  
 CAGGCCACACTGGAGGAGGCTGAGCAGAAAGAGGGGGAGTTTACAGCAGAT  
 5 GTTGGAGCAGTTGAAAGAGCAGCAAGAGGAGGCTCAGGCCGTGGCAGCTG  
 CCTCAGCAGGCTCAGAGACTTACAGTGGGATAGGAGGGTTAGGAGAACTT  
 CTGGAGAGTTCTCAGAACGCTTCAAGTTGAGCTCCAAGAGTGCCAAGGA  
 GTGAGGAATTCGAGGAAGAAGAGGAGACAGGGGAGCACTTGAGGGAA  
 ACCACAGACCCGAAGGAGAACAGGTTCCTCCAAAGTCGGAATCAGAACAGC  
 10 GTCAAGCAGAAGGAGTTCTGTTCTCCCTGGATGGAACCCCTGAGCGG  
 CGACAAAGCTGTCCTCCCATCAGTCTCTTGAGTATCCTGGCT  
 CCCCCTTCCCCAACGAGCAATAGCAAAACGAGCATTTTCAGCTTCAGA  
 GGTGGCGGAAGGAGCTGGGGTCTGAGAATGACTTTGCGGATGATGAA  
 CAGCACCTTGAAGATGAGCAGGACAGGGAGAACACTACTGTTTGTGGCG  
 15 ACAGACCTGGAGAGGAGCAGCAACAGTAACGTTAGTCAGGCCAGTATGTCA  
 TCCAGGATGGTCCAGGGCTTCCAGCCAATGGGAAGATGCAACAGCACTGT  
 GGATTGCAATGGTGTGTTCTGGTACACCACTGAAACAGAACAGTCA  
 GGAAGAGAACGGCTAAGTTTACCAAGTCTGATGGATGAAARTGTTGGAGGAT  
 TCCCTGGGGACAAAGAGCCATGAGCATGCGAGTATCTGACCAACAC  
 20 GATGGAGGAACTTGAAGATCTAGACAGAACAGTGTCCACCATGCTGGTATA  
 GATTGCAATGTTGTTTGATGTTGGACTGGTGTGATTCTGGTGTGAA  
 GTAAAGCATCTTGATGAAATTGATGTTGATGATGATCCATTGTCGACCTGG  
 CATCACCATCTGATGTTAACACACATGTTTACATGGCCATGGAGCACT  
 ACCCGATGAGGAGCAGTTCACAGTGCTGACGGTGGGAAACCTGGT  
 25 TTACCGGGATCTTCACAGGGAGATGGTCTGAAAATCATGGCAATGG  
 TCCCTATTACTATTTCAAGGGCTGGAAATATCTTGATGGAATTATTG  
 TTAGGCTGAGTTTAATGGAGCTGGCTGGCTGGCAAACGTTGGAGGGCTGTG  
 GTGCTTCGGTCTTCAGACTGCTGAGCTTCAAGTTGCAAATCCTG  
 GCCCACACTGAAGATATGCTTAAAGATCATGGCAACTCGGTGGCGCAC  
 30 TGGCACCTGACCCCTGGCTGGGACATCATGCTTCTCATTTTGGCGTG  
 GTGCGCATTCAGCTGTTGGAAAAGACCTACAGGAGTGTGTTGCAAGAT  
 CAATGAGGACTGCAAGGCTCCCGCCTGCCACATGAACGACTCTTCCACT  
 CCTTCTGATGTTCCCGCTGTGTTGGGAGTTGATGAGAACCATG  
 TGGGACTGCTGGAGGCTGGGGGACACATGTCCTTATTGTT  
 35 GTGGTCACTGTTGATGTTGGAACCTTGTGTTCTGAACCTCTCCCTGGCCT  
 TATTGTTGAGTTCTTGTGTTGACAAACACTGCTGCTACGGAGATGAT  
 AACGAATGAAACACCTTCAGATGCCGTTGGGAAAGGATGCAAAAGGGAT  
 TGATTATGTAAGAAAATAGATAACGGGAGTGTCTCCGAAAAGCGTTTTTA  
 GAAAGCCGAAAGTGTGATGAGAACATCAGGAAAGGGAAACAAATAGACAGCTGC  
 40 ATGTCCTAAACACGGGGCTAGTTGAAATAAAGCAAAGGCTTAAACTACCT  
 TAAAGACGTTAACGGAACCCAGTGGCGTGGGACTGGAAGCAGTGTG  
 AGAAAATCTTAATTGATGAAATGACTACATGTCATTCAACAAACCCC  
 AGCCCTACCGTGAACGGTGGCAATTGCGCTGGGAGGACTGCACTTTGAAA  
 TTAAACACGGAAAGAGTTAGCACTGACTCAGAACTGGAAAGAACAGAAC  
 45 AGAAAATTAATGCAACCGACTCTCTGAAGGAAGGACAGCTTGACCTGG  
 CGGGCCCGAGAACAGTGAACACAGCTGGAAATTGAACTCTGAGGAGGACCTTA  
 GCCAGAAAGCTGCTTACTGAAGGATGCAATTAAAATTTCCCTCTGCC  
 AAGTAAGTAGGAAAGGAAGGTAAAGGAAAATCTGGTGGAACTTTAGGAAG  
 ACCTCTTATGATTGTTGAAACAACACTGGTTGAGACCTTCTGATTGTT  
 50 CATGATTCTCTCAGTAGTGTGCTTGGCTTGGCTTGAAGATATACATTG  
 AGCAACGGAGAACCATCAAAACCATGCTGGAGTATGCTGCAAAGACTCTC  
 ACTTACATCTCATCTGGAAATGCTCTCAATGGTGGCTTATGGATT  
 TCAAACCTATTTCACCAATGCTGCTGGTGTGGACTTCTGATTGTT  
 ATGTTCTTGGTGAAGCTGGCAACGGCTCTGGCTATTGAGAACACTT  
 55 GGTGGCCATCAATCCCTACGGACCCCTGAGAGCTCTGAGGGCCCTCCGAGC  
 CTTATCCCGTTGAAGGATGAGGGTGTGTAACGCTTGTGCTTGTG  
 CAATCCCCCATCATGAATGCTACTGGTGTGCTCATCTTGTGTTA

ATCTTTAGTATCATGGGTGAACTGTTGCTGAAAGTTCTATCACTG  
 TGTTAACATGACAACGGCAGCATGTTGCACATGAGTGAAGTCACAAATT  
 TCAGCGACTGTCAGGCTCTTGGCAAGCGAACGCCGATGAAAGAATGTGAAA  
 GTCAACTTGCACATGTTGGGCTGGTACCTGGCATTGCTGCAAGTGGC  
 5 CACATTCAAAAGGCTGGATGGATATTATGATATGCACTGTGGATTACCGGG  
 ACCTCAAACTGCAAGCTGTATATGAAGAAAATCTGTACATGTTATCTGTAC  
 TTGTCATCTCATCATCTTGGGCTGTTCTACTCTAAATCTATTCAT  
 CGCGACTCATGACAGAACCTCAACCCAGCAGAAAGAAGTGGAGGTC  
 AAGACATCTTATGACAGAAAGAGCAGAAAAAGTACTACATGCAATGAAG  
 10 AAACCTGGCTCAAAACACCTCAGAAGCCATCCCTGACCTGCAACAA  
 ATTTCAGGAATGTCCTTACTGTTAAACAGACAAGTGTGGACATCA  
 GCATCATGATCTCATGTCCTCAACATGGTGGACCATGATGGTGGAAACG  
 GACGACAGAGCAAAATCACATGACCTGTTGTCCTGCAAGCTCATCTCTCA  
 15 ATTCATGCTGCTCTTCATGGGAGATTTCTGCTGAAGCTCATCTCTCA  
 GATACTACTACTCAGGTTGGCAGAACATCTTGACTTTGTTGGTGGTG  
 ATTTCCTCATGTTAGGAAATGTTCTTGTAGCTGATAGAGAAAGTATT  
 TTGTCATGCTTCTTACCTGTCAGGAGTCTCCGCTGGCCAGGATGGACGAA  
 20 TCTCACCCCTGATCAAGGGCAGGGGATCCGGACGCTGCTCTTGGT  
 CTGATGATGTCCTCTGGCCCTGTTAACATCGGCCCTCTGCTCTTGGT  
 CGTCATGTTCATGTCAGGCTACATGTTGGGATGTCAACCTTGCTTATGTTA  
 AAAAGAGGCTGGAAATTGATGACATGTCACATTGAGACTTTGGCAAC  
 25 ACCATGATCTGCCATGTCACATGGGAGGACTCTGAGGCTGGGATGGACT  
 GTTGGCCCCCATCTCAACAGTCGACCTCTGACTGTGACCCCTGATGCAA  
 TTCACCCGGAAAGCTAGTGAAGGGAGACTGTTGGAAACCCATCTGTGGG  
 ATTTCCTTTTGTGATCATCATATCATATCTTCTCTGTTGTTGTTGAA  
 30 CATGTCATGCTGTCATCTTGGAGAATTCAGCGTTGCCACAGAAAGAAA  
 GTGAGAGCTGACCCCTGACGCCACCCAGTTCAATAGGTTCTGCAAGCTCTC  
 TGACTTGAGCTGCCATCTCCCTCATCGCAAAGCCAAACCA  
 35 AAGTCAGGCTCATGCCATGGACCTGCCCATGGTAGCTGAGACCCGATC  
 CACTGCCCTGGACATCTTATTTGCTTTACAAAGCGGGCTCTGGTAGAG  
 TGGAGAGATGATGCCCTCTGAAATCAGATGGAAGATCGTTCATGGCTT  
 CCAATCCCTCAAGGCTCTTATGAGCCATTACCAACCTCTGAAGGCGC  
 AAACAAAGAGGAGTGTGCTGCTATCATTCAGCTTAATCAGATGTTA  
 40 TCTTTAAAGCAAAAGTAAAAACATATCAAAATACGTATGACAAAGAGA  
 CAATGCAAGGGGAGGATGTTGCTGCTATAAAGGAGATATGTTATGAC  
 AAATTAATGGAAATTCCTCCAGGAAAGACAGATGGGAGTCTCTAC  
 CACCTCCCTCTTCCATGACAGTGTAAACAAACCAAGATAAGGAAAAGT  
 TTGAGAGAAAGACAACCCAGAAAAGGAAAGGAAAGGGAGTCTGAGAG  
 45 AATCAAAGATAATGTCCTTGTAACTATGTTACGCCCTGAGGTTAA  
 GTGTCGGTGTCAACTGGACTC

## 45 SEQ ID NO:4

Genbank® GI No.: 19923380; Ref No.: NM\_006922.2; Homo sapiens sodium channel, voltage-gated, type III, alpha (SCN3A), mRNA

AGCGAAGCGGAGGCATAAGCAGAGAGGATCTGGAAGGTTCTTTGTTCTTATCCACAGAGAAAGAA  
 50 AGAAAAAAATGTAACATTTGTAACCTCTGTTGCTAAAAAAGAAAAAAAGCTGAACAGC  
 TGCCAGAGGAACACGTTATACCTAACATCTGGATGCTGGCTTGTATGCTGTAATTCTCATAGG  
 CTCTGTTTATCAGAGATGAGGCAAGAAAATGAGGCAACGACATCAAGGTTGACAGGGATGAG  
 ATACATGTCAGGATTCTAGTAGAGTGTGCTACTGGAAAAGGAGCAAAGAATCTCTTAGGGATATTG  
 TAAGAATAATGAGATAATTCAAGAAGGGACCTGGAGCTTCCGAAAAGGTTGCTGTGACTATCTAA  
 55 GGTAAATTCTATGCAAGAAGCTACACGTAAATTAAATGTCAGGATGAAAAGATGGCACAGGCCACTGTGG  
 TACCCCGAGGACTGAAAGCTTCCGCCCTTTACTAGAGAAATCTCTGCTGATCGAAAAGCTGCTG





AAATATATATAATTAAACCTGTGCTTGATCTGACATTGATACATAAAAGTTACATGAATTITA  
 CAAACAACTAGTCGATGATTCAACAGCACTACAGAACAAAGGCAAAATTAAAAGCAGCTTGTGAAC  
 TTTTATGTGTCAGGATCAAGTTCATGTTCCAACTTCTGAGTTGATAATAATGAGTAAACCAC  
 TACAATAGCTTCATAATTCAACTCCCTGGCTATAAGCATCTAAACTCATCTCTTCAATAAT  
 5 TGATGCTATCTCTTAACTTGTGGCTATAAAATGTCATTTCTGTTACTTAAATGCAATTATAA  
 ACTCTATGATCATAAAGGTATAATGATAGTTAGTATGAGAATTATATAACTTTTTCAAGAAC  
 CCTTGGATTATGTGAGTCAAAACACTTCTCAGTGGAAAACCTCCAGTTGTAATGCAATA  
 10 TTAAAGACAAATTGGATCAAATATGATTTCTATAATTCTCCATAATAAATTATAAGGGAAAAAA  
 AAAAAAAAAAAAAAAAAAAAAAA

10

SEQ ID NO:5

GenBank® GI No.:19923381; Ref. No.:NP\_008853.2; sodium channel, voltage-gated, type III, alpha; sodium channel, voltage-gated, type III, alpha polypeptide [Homo sapiens]

15

MAQALLVPPGPESFRLFTRSLAAIEKRAAEKKPKKEQDNNDENKPKPNSDLEAGKNLPFIYGDIPP  
 EMVSEPLEDLDPPYINKTFIVMNGKAIFFRSATSLYILTPLNPVRKIAKILVHSLFSMLIMCTIL  
 NCVFMTLSNPWDTKNEYFTGIFTYTFESLILKILARGFCLEDFTFLRDPWNWLDFSVIVMAYVTFVDLG  
 NVSALRFTVPLRALKTISV1PGLKTIQVQMLSVMLTFVFCLSVFA1GQLQFLMGNRLRNKCLQW  
 20 PPFDSAFEFTNTTSYFNGTMDNSGTFVNTMFTNWKDY1GDDSHFYVLDQKDPPLCGNGSDAGCPEGY  
 ICVKAGRPNYGYTSFDTFSWAFSLFLRMLMTQDYWEFLYQLTLRAAGKTYM1FFVLVIFLGSFVYLNL  
 AVVAMAYEEQONQATLEEEAQEKEAQQMLEQLKKQEEAAQVAAAASRDFSGVGGLGELESSSEASK  
 LSSKGAKEWNRNKKRQREHLEGNNKGERDSFPKSESEDSVKRSSFLFSMDGNRLTSDDKKFCSPHQSSL  
 25 SIRGSLSPRPRNSKTSIFSFRGRAKDVGSENDFADDEHFEDGESRRDSLFVPHRHGERRNSNVSQASM  
 SSRMVPGLPANGKMHSFTDCNGVQGSPALSTPTQOLPPGGTTTETEVRKRRLSYYQISMEMLDSS  
 GRQRAVSIAISLNTMEELEESRQKCPWYRFANVFLIWDCCDAWLKVHVLN1VIMDPFVLDIAITICI  
 VLNTLFMAMEHYPMTEQFSSVLTVGNLVFTGIFTAEMVLK1IAMDPIYYFQEGWNIFDGIVSLSLMEGL  
 30 LSNVEGLSVLRSFRLRFLVFLAKSWPTLNLMIK1I1GNSVAGLNLNTLVLAI1VIFIAVFGMQLFGSKYE  
 CVCKVINDDTPLRWNHMDFFHSFLIVFVFLCGWEITWMDCEVAQGTMCLIVFMLVVMVIGNLVNLFL  
 ALLLSSSSDNLATDDNEMNNLQIAVGRMKGIDYVKNKMRRECQKAFRRPKVIEIHEGNKIDSCMS  
 35 NNTGIE1KESLNLYLRDNGGTTGVTGSGVEKYV1IDENYMSFINNPSLTVTVPIAVGESDFENLNTTEF  
 SSESSELEESKEKLNATSSSEGSTVDDVLPREQEATPEEDFPKPEACFTEGC1KKFPFCQVSTEEGKGK  
 IWWNLRKTCYSIVEHNWFETFIVFMILLSSGALAFEDIYIEQRKTIKTMLEYADKVFYT1FILEMLLKWV  
 AYGFQTYFTNAWCLDFLIVDVSLSVSLVANALGYSELGAIKSRLTRALRPLRALRSRFEGRMVRVNNALVG  
 40 AIPSIMNVLLVCLFLWFLIFISIMGVNLFLAGKFYHCVNMTGNNFDISDVNNLSDCQALGKQARWNKVNKF  
 DNVGAGYALLQVATFKGWMIDYAAVDSRDVKLQPVYEEENLYMLYFV1F1IFGSSFTLNLFIGVIIDN  
 FNQQKKKDF1MTMEEQKYYNNAMKKLGSKKPKP1PRPANKFQGMVFDVTRQVFDISIMIICLNM  
 VTMVETDDQGKYMFLVSLRSLN1FLVFLFTGEFLVFLKVLSLRHYFT1GWNIFDFVVVILSIVGFMFLA  
 EKYSVSPTLFRVIRLARIGRILRLIKGAKGIRTLFLALMMSLPAFLNIGLFLVMFYI1FGMSNFAYV  
 KKEAG1DLMFNFETFGNSM1CFLQ1ITTSAGWDGLLAPILNSSAPDCDPDTIHPGSSVKGDRGDPVGF  
 45 FVSYI1ISFLVNVNMYIAVILENFSVATEAEP1SEDDFPEMVEWEKFDPDATQF1EFSKLSDFAAAL  
 DPPLLIAKPNKVQLIAMDLPMSVGDRIHC1DILFAFTKRVLCESGEMDALRIQMEDRFMASNPSKVSEYP  
 ITTTLKRKQEEVSAAI1QRNFRCYLLQKRLKNISSNNYNEAIKGRLPIKQDMIIDKLNGNSTPEKTDG  
 SSSTPPPSYDSVTKPDKEKPEKDKPEKESKGKEVRENQK

45

SEQ ID NO:6

GenBank® GI No.: 12642271; GB No.:AF225986.1; Homo sapiens voltage-gated sodium  
 50 channel alpha subunit splice variant SCN3A-s (SCN3A) mRNA, complete cds, alternatively  
 spliced

AGCGAAGCGGAGGCATAAGCAGAGAGGATTCTGGAAAGGTCCTTTCTTCTTACACAGAGAAAGAA  
 AGAAAAAAAAATGTAACATAATTGTAACCTCTGTGGTCAAAAAAAAAAAAAAAAGCTGAACAGC  
 55 TGCCAGAGGAAGACACGTATACCCCTAACATCTGGATCTGGCTTTGTATGCTGAATTCTACAGG





5 CAAACAAAAGTTAATGTTAATCTTTACAAAACATTACTGTAGTGTATTGAAGAACATGCAGCA  
 GGGAACTGCTATTGCTAAAGAAAGTGTGACTGCTGAGCTTATTGAGCCTTAAAGGAAAGAAATAAATTCATTTT  
 TATTGCTATTGCTCTTCTGGGGCTTTGTTTTGTTTTGTTCTGTTGCTGGCAGTTAACAAAA  
 10 TATAATTAATAAAACCTGTGCTTGATCTGACATTGTATACATAAAAGTTACATGAATTACAACAAA  
 CTAGTCATGAGTCACCAAGCAGTACTACAGAACAAAGCCTTAAAGCAGCTTGTGAACATTATG  
 TGTGCAAAGGATCAAGTTCACATGTTCAACTTCAGGTTGATAATAATAGTAGTAAACCACTACAATA  
 GCTTCAATTCAATTAACTCCCTGGCTATAAGCATCTAAACTCATCTCTTCAATAATAATIGATGCT  
 ATCTCTAACTTCTGCTCTTAAATAGTTACATTCTTGTACTTAAATGCTATTATAAACTCCTA  
 15 TGTATACATAAGGTTAATGATATAGTTAGATAATTAACTTACAGGTTAATGCTATTAAAGGAACTCCAGTGTAA  
 TTTATGTGAGGTCAAACCAACTCTTATTCTCACTGGAAAACCTCCAGTGTAA  
 CAATTGGATCTAAATATGTTCTACATAATTCTCCATAATAAATTATAAAGTGGAAAAAAA  
 AAAAAAAAAAAAAAAA

## 15 SEQ ID NO:7

GenBank® GI No.:12642272; GB No:AAK00218.1; AF225986\_1 voltage-gated sodium channel alpha subunit splice variant SCN3A-s [Homo sapiens]

20 MAQALLVPPGPESFRLFTRESLAAIEKRAAEKKPKKEQDNDENKPKPNSDLEAGKNLPIYGDIPP  
 EMVSEPLEDLDPPYINKFTIVVMNKGKAIFFRSATSAVYIILPLNPVRKIAKILVHSLSFSLMILMCTILT  
 NCVFMTLSNPDPWTNKVNVEYTFGTGIFTESLIKILARGFCLEDFTLRDPWNWLDFSVIVMAYVTEFVDLG  
 NVSALRTFRVLRAKLTISVPIGPKLTIVGALSVKLLSDVNILTVPCLSVFALIGLQLFMGNKLCQLQW  
 PPSDSAFETNTTSYFNGTMSNGTFVNVTMSTFWNKDVYIGDDSHFYVLDGQKDPPLCGNGSDAGCPEGY  
 25 ICKVKAGRPNYGYTSFDTFSWAFLSLFLRMLTDQWENLYQLTLRAAGKTYMIFVFLVIFLGSFYLVLNLL  
 AVVAMAYEEQNQATLEQEEKEAEEFQQMLQLKKQKQEEAAQVAASASRDFSGVGGLGEELLESSEASK  
 LSSKGAKEWNRNRRKKRQRREHLEGNNNGERDSFPKSESEDSSVKRSSFLFSMDGNRLTSDFKKCSPHQSSL  
 SIRGSLSPRPNRNSKTSISFRGRAKDVGSENDFADDEHSTFEDSESRRDSLFPVPHRGERRNSNGTTET  
 EVRKRRRLSSYQDLESDSSGRQRAVISIAILTNTMEELSERQKCPCPWYRFANVFLIWDDCCANLKV  
 30 KHLVNLIVMPDPFDLAIITCIVLNLTMFAHEHYPMTEQFSSVLTGVNLVFTGIFTAEMVLKIADMPVYY  
 FQEGWNIFDGIIVSLSLMEGLSNVEGLSVLRSFRLLRVKLAKSWPTLNMLIKIIGNSVAGLNLTVL  
 AIIVFIVFAVVGKSYKECVCVCKINDCTLPRWHMDFFHSFLIVFVRLCGEWIETMNDCMVEAGQTM  
 CLIVFMLVMVIGNLVLNLFLLALLLSSFSDDNLATAADDDNEMMNLQIAVGRMQRKGIDYVKNKMRRECQKA  
 FFRKPKVIEIHEGNKIDCSMSNNGTIEISKELNLYLRDNGNTTSGVGTVGSVEKYVIDENDYMSFINNPSL  
 35 TVTVPIAVGESDFENLNTFESELEESKEKLNATSSSEGSTVDVVLPREGEQAEPEEPEDFPKPEACF  
 TEGC1KKFPFCQVSTEEGGKINWNWLRTKCVSIEHNNWFETIVFVMISSGALAFEDIYIEQRKTIKTM  
 LEYADKVFVTYIFILEMILKWKVAYGFQTYFTNAWCMLDFLIVDVSLSVLVAANALGYSELGAIKSLRTRLR  
 RPLRALRSFEGMVRVVAUNLNGAIPSIMVNLVCLFLWIFLTSIMVGVLNFLAGKFYHVCMNTIGNMFDISDVN  
 40 NLSDCQALQKQARWNKVNKVFDNVGAGYLLALQQVATFKGWDMDIMYAAVDSRDVKLQPVYEEENLYMLYFV  
 IFIIFGSSFTLNLFIGVIIDNPQNQKKFFGQDIFMTEEEKKKYNNAMKKLGSKKPKQKPIPRPANKFQGMV  
 FDFVTRQVDFISIMILCILNMVNTMVTEDDGKYMVLVLSRINLVFIIVLFTGEFVFLKLVLVSRHYFTGW  
 NIFDFVWVLLSIVGMLFAEMIEKYSVSPTEFLRVRILRIGRLRIGKAGKIRTLLFALMMSLPALPNIG  
 LLLFLVMFIYIAIFGMSNFAYVKEAGIDDMFNFTFGNSMCLFQITTSAGWDGLAPILNSAPPDCPD  
 TIHPGSSVKGDRDPSVGFIFVFFSYIIISFLVVMYIAVILENSVATEESEAEPPLSEDDFEMWEK  
 45 FDPDATQFIEFKSLDFPAALDPPLIAKPNVQLVIAIMPLVMSVSDRHLCLDLIFAFTKRVLCESGMDA  
 LRIQMEDRPMASNP SKVSYEPITTLKRKQEEVSAIIQRNFRCYLLKQRLKNISSYNKEA1KGRIDL  
 IKQDMIIIDKLNGNSTPEKTDGSSSTTSPSPSYDSVTKPDKEKFDKPEKESKGEKVERENQK

## 50 SEQ ID NO:8

GenBank® GI No.:6981509; Ref. No.:NM\_013119.1; Rattus norvegicus sodium channel, voltage-gated, type III, alpha polypeptide (Scn3a), mRNA

55 CAGTGTGTTTGTGCGCAATGGCGTTGTTCTGCGAGTAGTGTGCACTGAGCAGCTGAGTGTGAGTCGGCAA  
 CCTTTCTCTTTCTCTTTCTCTTCTCTTCTCTTCTCTTCTCTTCTCTTCTCTTCTCTTCTCTTCTCTTCTCTTCT  
 CATAGAGTGAATCTGAGAACAGGAAGGGCAGGCTAAGCAGAGGAGGATTCGGAAAGGTCTTCTTGTGTT  
 CATGTCCACAGAGAAAAGCAAGGGGAAAAATTGAATGTAAATTGCAATCCCTGTGGCCCAAATCTGAAG



CATGAATGTGTTATGGGTGTCATCTCTGGCTGATTTAGCATCATGGGTGTAATCTGTTGCT  
 5 GGAAGTCTTCTACTGTTAACAGCACAGGCAACTGTTGAAATAAAAGAACAACTTCTCA  
 GTGACTGTGAGGCTCTGGCAAGGACGCCGGTGAAGATGTGAAAGTCATCTTGCACAGTGGGGC  
 TGGCTACCTGGCATGGCTGAAGTGGCACATTCAAAAGGCTGGATGGACATCATGTATGCACTGTTGAT  
 TCGCGGAGCTCAAACCTGAGCCCATATGAAGAAAACCTGATCATGTCATGTTGCTCATCTCA  
 10 TCACTCTGGCTCTTCTACTAAATCTATTGATCATGGTGCATCATAGACAACTTCACAGCAGAA  
 GAAGAAGTTGGAGTCAGACATCTTATGACAGAAAGACAGAAGAAATACTACAAATGCAATGAAGAAG  
 CTGGCTCAAGAACCTCAGAACGCCATCCTGCCGCTGCAAAACAAATTCAAGGGATGTTGCTTTGATT  
 TTGTAACACAGAACAGTTGGACATCAGCATGATCTCATGCTCAACATGGTGACCATGATGTT  
 15 GGAAACGGATGACCGAGAGCAAATACATGACCCCTGGTTTGTCCGAATCACCTAGTGTTCATGTCCTC  
 TTCACTGGGGATTTCTGCTGAACTCTCCCTCAGATACTTACACTACTACTTCACCGATAGGGTGGACACTT  
 TTGACTTTGTGGTGTGATTCTCTGCTGATTGGAGATTGTTCTGCAAGCTGATAGAGAAGTATTCTG  
 20 GTCCCTACCCCTGTCAGTCAGCCTGCCAGGATTGGACGAATCTACGCCCTGATCAAAGGCC  
 AAAGGGATTCGGCACTCTGCTCTTCTGTTGATGTTGCTCTGCCGCTGTCACATCGGCCCTCTG  
 TTTCTGGTGTGATCTACCCATCTGGGATGTCACACTTGTCTATGTTAAAAAAAGGGCTGG  
 AATTGATGACATGTCACATTGAGACTTTGGCAACAGCATGATCTGCTGTGCTTCAAATCACCACCTCT  
 25 GCGGGCTGGGAGCCTGCTGGCCCCATCTCCACAGGCCACCTCCGACTGTGACCCGATGCAATT  
 ACCTCTGGAAAGCTCTGGTAAAGGGGACTCTGGGAAACCATCTCGTGGGAGATTCTTTTGTCACTACAT  
 CATCATATCTTCTGGTTGGTGAACATGATCATGCTGTCTGACCTGAGAATCTCAGCTGCCACC  
 30 GAAGAGACAGCTGGAGGAGCTGAGTGGAGGACTCTGGAGATGTTCTACGGAGGCTGGGAGAAAGTCTG  
 CTGACGCCACTCAGTCTAGAGTCTGCAAGCTTCTGACTTCTGAGCTTCTGAGCTGCCCTGGATCTCCCT  
 CATCGCAAAAGCAAACAACTGAGCTCATGGCATCTGGACTCTGGTGTGAGGAGCCGATCTCAC  
 TCCGCTGACATCTGTGTTGTTTACAAAGGGCTCTGGGGAGAGTGGAGAGATGACCCCTCTGAA  
 35 TCCAGATGGAGATGGCTCTCATGGCTTCAACCCCTTCAAGGTTCTTATGACCCCATACCAACCCCT  
 40 GAAACCGAAAACAAGAGGGGGTGTGCTGCTGCTGATCATTCAGCGTAATTATGAGATGTTATCTTAAAGCAA  
 CGGTTAAAAAAACAATGCGATAATAACGACAAGAGACATAAGGGGAAGGATGACTTGCCTATAAAAG  
 GAGATATGGTTATTGACAATTGAAATGGGAACTTCACCCAGGAAAGACGGGATGGGAGATTCCTCCAAAC  
 45 CTCCTCTCTCTATGACAGTGTAAACAAACCGATAAGGAAAGATTGAGAAAGACAAACCGAAA  
 GAAATCAAAAGGGAAAGGTCAGAGGAGATAAAAGATAAAAGGACAAAGGAAATGCTTTGTAATCAAT  
 TGTTCACCTCTGGTGTAAAGTACCTGGCTCAACTGGACTCTAGAGGAGGCTGCCATGCCCACACTGAC  
 50 AGGGTCAACATGGCAAGAGGTTGCTGCTCTCCATTACAGCTGCAACTGCTGAGGAGAGCTCCATG  
 GCAAGTGACCGTCACTGCCCAAACTTGTACAGCTCTGGTGTGATCTTAACTTCATGTCATGTT  
 CATTGTCATATTTCACAAATTCAGTGTGCTGTTCTGGTCCCTCATGTCATGTCATGTT  
 55 TACTATGTCACTATTGGTAAATGAAGTTACGTAAAGGAAAATATAATATAAGAATCCATGTC  
 TAAGTCCACAAGTTCTCAGTAATCATAAAAAAATATTGTCAGAGATGAAATTATTGTCAAAAC  
 AAAAAAAATAATTCTAATGTTAACAGCTTC

## SEQ ID NO:9

GenBank® GI No.:6981510; Ref. No.:NP\_037251.1; sodium channel, voltage-gated, type III, alpha polypeptide [Rattus norvegicus]

MAQALLPPGPESFRLFTRSELAAIEKRAEAKKPKKEQIDDENPKPKPNSDLEAKNLPFIYGDIPP  
 45 EMVSEPLEDLDPPYVSKTFVLNKGKAIFRFSATSAVLYLTLPLNPKRVIKILVHSLFSMLIMCTIL  
 NCVFMTLSNPDPWTKNVVEYFTGIYTFESLILKILARGFCLEDFTLRDPWNWLDFSVIVMAYVTEFVDLG  
 NVSALRTFRVLRAKLTISVPGKLTIVGALIQSVKLLSDVMILTVFCLSVFALIGLQLFMGNLRNKCQSOW  
 PPSDSAFEFTNTTYSFNGTMDNSGTFVNVTMSTFWKHDYIADDHSFYVLDQKDPLLCGNGSDAGQCPBEGY  
 50 ICVKAGRNPNYGTSDFTSWAFLSLFLMTQDYWENLYQLTLRAAGKTYMIFFLVLFILGSFYLVNLIL  
 AVVAMAYEEQNQATLEEAQEKEAEEQFQQMLLEQQLKKKQEEAQAVAAASARDFSGIGGLGELESSSEASK  
 LSSKSAKEWRNRRKKRQRREHLEGNHNRADGDRFPKSESEDVKRRSFLLSLDGNPPLGDKKLCSPHQSSL  
 55 SIRGSLFSPRRNSTSISFSFRGRAKDVGSENDFADEHSTFEDSESRRDSLFPVRPGERNSNGTTET  
 EVRKRRNLSQYQISMEMELEDDSSGRQRSMSIASLTNTMEELEESRQQLCPCCWYRFPANFLWIDCCDAKLKV  
 KHLVNLIVLMPDPFLAITICVLNTLFMAMEHYPMTQPFQSSLTVGNLVTGIFTAEMVLKIAIMDPYYY  
 FQEGWNIFDGIIVSLSLMELGLANVEGLSVLRSFLLRVPFLAKLSWPTLNMLIKIIGNSGVALGNLTLVL  
 CLIVFMLVMVIGNLVLVNLFLALLSSFSDDNLAATDDDEMNNLQIAVGRMQKGKIDFVKNKIRECFRKA

FFRKPKVIEIQEGNKIDSCMSNNTGGIEISKEELNYLKDGNGTTSGVGTGSVEKYVIDENDYMSFINNPSL  
 TVTVPIAVGESDFENLNFSEESKEKLNSSEESTVVDVAPPREGEQEIAEPEEIDLKPEACF  
 TEGC1IKFFPFCQVSTEEGKGKINNLRKTCVIEHNWFETPVIMILLSSGALAFEDIYIEQRKTIKTM  
 LEYADKVFVTFYIIFILEMLLKWKVAYGFQTYFTNAWCWLDFLIVDVSLVSLVANALGYSELGAISLRLRAL  
 5 RPLRLSRSFEGMRRVVAALNGAIPSIMVNLVCLLIFLWISFTIMGVNLFAKGKFYHCVNNTTGNMFEIKEVN  
 NFSDQALQKGQARWNKVNKVNFNDNVGAGYLALLQVATFKGWMIDMYAAVSDRDVKLQPIYEENLYMLYFV  
 IFPIIFGSPFTNLFIGVIDNPNQOKKKFFGQDIFPMTEEQKKYNNAMKKLGSKKPKPIPRPANKFQGMV  
 FDFVTRVQVLFISIMILCNCMVTMMVETDOSKYMTRLVLSRINLVFVILFTGFELLKLIISLRYYFTIGW  
 10 NIPDFVVVLISIVGMPFLAELIEKYFVSPPTLVRIRLARIGRILRKIGAKGIRTLIIFALMMSLPAFLPNIG  
 LLLLFLVMFYIAIFGMSNFAYVKEAGIDDMNPNFETFGNSMCLFQITTSAGWDGLLAPILNNSAPPDCPD  
 AIHPGSGVKGDCGCPNSPVGIFFFVSYIISIISFLVVMYMIAVLENFSVATEESEAELPDDFEMFYEVWEK  
 FDPDAQTFIEFCKLSDPAALDPPLIAKPNQVLIAMDPLPMVSGDRIHCLDLIFAPFTKRVLGESEMDA  
 LRIQMEDRFRMASNPSKVSYPEITTLRKQEEVSAAIICRNYRCYLLQQLRKNISSSYKDKEITKGRIDL  
 IKGDMVIDKLNGNSTPEKTGSSSTTSPSPSYDSVTKPDKEKPKEDKPEKEIKGKEVRENQK  
 15

SEQ ID NO:10]

GenBank® GI No.:9055329; Ref. No.:NM\_018732.1; *Mus musculus* sodium channel, voltage-gated, type III, alpha polypeptide (Scn3a), mRNA

20 TTTTATATCTGTGAGGGTACGAAAGAAAAAGAAAAAGAAAAAGAAAAATGCAAATAAAATGGAG  
 CCCAGTAAATGTCAGGATTTAATCAATTCAATTCTTATTGGAGGAACACTTTAAAGCATGTAGAAGC  
 TGAGGATCTGGTCATCTCAACACCCGACTTGGGGGAGTGAACGGCAGAAGAAACTAGCTGATTATAC  
 TCTCGATAGTATTTGAGTCTGGCTGGGCTACATGAAACTGTATCTCATCCAAACACATAAAA  
 AAGTTGATATTTGATATTTAAACAAATGATAACAAATGTTAGAAATTATAGCAAACAAATAATTGG  
 25 TAAAAGTACCTTACATATATATATATATATATACATATACATATATATGATATACATATACATATAT  
 GTATTTTTAAATTTAAACAAAGCTTTAAACATGAAATTCTTACCTTCACTTTAAAGAGAAATGAAATTCA  
 TATTTCACACATTCTTCAGTTCTAGAGTGAATCTGAAGTGACAGTGTAGCATTTAGTAGTGTCTGTT  
 GTGTCACATCTGTCATCCACCCAGCTTGGCCACCTGTAAATTAGAGACCCCTTCAACAGGCAAG  
 GGGCCAGGGTCTGGCGCAGCTGCTGAAATTAAAGTATTAAATTCTTAGGTGATCTAAA  
 30 AGTAAATCTAAAATAGATTATAAAGAAAAATGTCTGAAATAACGACTTGTATTACACCCATAA  
 TTATTCTACCTTTTTGGAGCTACATTATGATCACTACATCCTTAAATAATAGTCTAGCTTCCAAAGTG  
 TACTACATTTCTTCTTATTCTCATACAGCTGGAGCTTCAGTGGCAAGTTGGCAAATCCTGGCCCAACTG  
 ATGCTCATTAAGATCATCGCAACTCGGGCGACTGGCAACCTGACCTGGTGCTGGCATCATCG  
 TCTTCATTTCGGTCTGGCGATCGCAGCTGGTGGAAAGAGCTACAAGGAGTGTGTTGCAAGGCTAA  
 35 CGAGGACTCAGAACCTCCGGCTGCGACATGAAACGACTTCTTCACCTTCTGATAGTGTCTGGCTG  
 CTGTTGGGAGTGGATAGAGACCATGTTGGACTGCATGGAGTCGGGGCAGACCATGTCCTTATG  
 TTTTATGTTGGTCTGGTGTGTTGGACCATCTGGTTCTGGCTGACCTTCTGGCTTATTTGAGTTG  
 CTTTAGCTCAGACAAACCTCTGCTGCTACGGAGCTATAAGCAACCTCCAGATCGGGTGGAGA  
 AGGATGCAAAGGGGATTGATATTGTAAGAAATAAGACAGGAGTGTCTCCGAAAGCCTTTTGTGAA  
 40 AGCCGAAGTGTAGAAAGATCCCAAGGAAACAAATAAGACAGCTGGCTCATGTCATGTTCAAA  
 TAAATAAGCAAGGACTTACACTTAAAGACGCTTACCTTCACCTGGCAACCCAGTGGCTGGGTACTGG  
 AGTGTGAGAAAATACGTTAATGTAAGAAATGACTACATGTCATTCTCATCAACACCCAGCTCCAGCTGA  
 CGGTGCAATTGGCTGGAGGAGCTGTCTGGTTGAAATTTAAACACGGAAAGAGTTTAGCAGTGA  
 ACTGGAAAGAAGACAGGAGTAAAGCGCTTCTGCTGAGCTGGCCACACTTTTGCGCAATCTACAGTA  
 45 TGGCTCTCTGATTGTTTTGGCTGTGAGGAGTCTTAACAGGCTTACATGCTGTTAAATTAAC  
 CTCAGGAGTAAAGTGGAAACTCATGAGAATGGTAAAGTGTGTTAAATTAACCTGGCTGGGTACTGG  
 AACGTAGATAAGTGGAAACTCATGAGAATGGTAAAGTGTGTTAAATTAACCTGGCTGGGTACTGG  
 CACAATGAGGCTTACATGTTCTGATTAACATGAGTAAAGCTGATTAAATGCAATCTCAAC  
 AAAAGAGATACTTTAACACATTTCCTCGCTAGTTGTTAGAAAGAACTCAATGAACTTATTA  
 50 TTAATGGGGTATGGCTACACTGTGAAAATTACATGTTGTTTACATGTTTACATGTTTAAAGTGT  
 ATTGTTTACTAGGAAGGGTCAAGGCTACATGTTGTTTACATGTTTACATGTTTACATGTTTAC  
 TTTCATCATACCTTAAAGAACTGAAAGTAAACATTGATGATTATAGTGGATCTACAGATGGGAA  
 ATTTCCGTATAGTGGAGTGTGCAAGTAAAGAGAGAAAGAGATACTTACATGCTGTTGAGCTATTGG  
 GTCTAATGACAATAGCTTGTGACATTACCTTCATATTATTTATGTTATGGCTTGGATGT  
 55 AACAGCTGGAGGGTTAATCTAGCACAGATGGTGTATAAAGGATCTCTAGCTGGAGGGAACTCAAC  
 CTGGAACAGACCAATGATGTTGTTATGGATTCTCTGGAGCTTGTGAAACAGCTACACCAAGAT

AATTTGCTCTAGAAATCCTTATTAGAAGTTATGGCATCACAACTCACAGTGGTCCCATTCTTTCATAT  
 TTTAGAATATGTTAAAACATAATGACAAAATGAGGTAGCAATGTTTATTTTACAGTTGC  
 5 AACTAAATGTCATAGCACATCTAATTCACTGTTCTCATCTAGTAGCTAAGACTCAATTCTGGCTTTA  
 TTTCTATCACCTGGCTAGAATTGACTTACAAGGAACCTAGAGGACTAGCAGGATTCACTGACTAT  
 TTGCTCAATTGGCTAAAGACAGTCTTATCAACTGGTCCCCAACAAACTCATAAAGTCATTAAAACCTC  
 TATTAAATTGATCTTACAGGCCGGTGTAAATTCTTAGGGCTCATATTGGGTACACATCGTTAAAAGT  
 AGAAAATTGTAATAGGCCCTCATATTGGGTACACATCGTTAAAAGTGGAAAGATACTGTTCTCCAAAGGA  
 10 GAGCCAGGGTTGATAATGACTCTCTGTTACTCAGGCCGAGTGGAGAGACAGGGCAAAGGGATGTGC  
 CGGTGTCCTGGAAAGCTCAGACTGCACCTGCGAGACTAACCTGGAGCTTGGAAATTGTCATGGTCA  
 CGAAGGAGTGCTCTAAACAGAACATTAGAAAGGGGTACCTAGGAACATCTGTTATAAAGGATAAA  
 GGACGTTAAGACTGAGAGATAATGGAGATTAACTTACACACTTATAGCAATGAGATGGTGAATTCTGG  
 GCCAAAATTAATAGAGAACCAAGCTAATTGCAATTACAGACCTCAAGAACTGAGGAATCAGAAAGT  
 GGCAATTCCACTGTCGATAAGGGCTTCTGAGGAAGGTACCCAGGTTGTATGGAAAGGAATTG

15 SEQ ID NO:11

GenBank® GI No.:9055330; Ref: NP\_061202.1; sodium channel, voltage-gated, type III, alpha polypeptide [Mus musculus]

20 MLIKIIGNSVGALGNLTLVLAIIVFIFAVVGMQLFGKSYKECVCKINEDCKLPRWHMNDFFHSFLIVFRV  
 LCGEWIETMWDCMEVAGQTMCILIVFMLVMVIGNLVVNLFLALLLSSFSSDNLAAATDDNEMNNLQIAVG  
 RMQKGIDYVKNKIRECFRKAFFRKPKVIEIHEGNKIDSCMSNNTGVVEISKELNLYLDGNGTTSGVGTGS  
 SVEKYVIDENDYMSFINNPSLTVTVPIAVGESDFENLNTEEFSSSELEESKEVSAFLFSSGHFCAIYTV  
 WLCLTVFCL

25

1 **WHAT IS CLAIMED IS:**

- 2 1. An isolated sodium channel type III  $\alpha$  subunit (mNa<sub>v</sub>1.3  $\alpha$  subunit) polypeptide,  
3 wherein the polypeptide comprises the amino acid sequence of SEQ ID NO: 2.
- 4 2. The polypeptide of claim 1, wherein the polypeptide essentially consists of the amino  
5 acid sequence of SEQ ID NO: 2.
- 6 3. An isolated mNa<sub>v</sub>1.3  $\alpha$  subunit polypeptide comprising at least 10 contiguous amino  
7 acids of SEQ ID NO: 2, wherein the polypeptide includes one or more of the  
8 following amino acids: isoleucine 289, proline 518, serine 728, serine 1355,  
9 asparagine 1909, threonine 1910, and valine 1921.
- 10 4. An isolated mNa<sub>v</sub>1.3  $\alpha$  subunit nucleic acid molecule that encodes the polypeptide of  
11 any of claims 1-3.
- 12 5. The nucleic acid molecule of claim 4, wherein the nucleic acid comprises the  
13 nucleotide sequence of SEQ ID NO: 1.
- 14 6. The nucleic acid molecule of claim 5, wherein the nucleic acid molecule consists  
15 essentially of the nucleotide sequence of SEQ ID NO: 1.
- 16 7. The nucleic acid molecule of claim 4, wherein the nucleic acid is an allele of the  
17 nucleic acid sequence of SEQ ID NO: 1.
- 18 8. A fragment of the mNa<sub>v</sub>1.3  $\alpha$  subunit nucleic acid molecule of claim 4, wherein the  
19 fragment encodes one or more of the following amino acids: isoleucine 289, proline  
20 518, serine 728, serine 1355, asparagine 1909, threonine 1910, and valine 1921.
- 21 9. An expression vector comprising the mNa<sub>v</sub>1.3  $\alpha$  subunit nucleic acid molecule of  
22 claim 4, operably linked to a promoter.
- 23 10. A host cell comprising the nucleic acid of claim 4.
- 24 11. An agent which preferentially binds to the mNa<sub>v</sub>1.3  $\alpha$  subunit polypeptide of claim 1.
- 25 12. An agent which binds selectively to the mNa<sub>v</sub>1.3  $\alpha$  subunit polypeptide of claim 1  
26 and not to a sodium channel type I or type II  $\alpha$  subunit polypeptide.
- 27 13. The agent of claim 12, wherein the agent is a small molecule, a nucleic acid, or a  
28 protein.

29        14. The agent of claim 12, wherein the agent modulates a mNa<sub>v</sub>1.3  $\alpha$  subunit polypeptide  
30            activity.

31        15. The agent of claim 13, wherein the agent is an antibody or antigen-binding fragment  
32            thereof.

33        16. The agent of claim 15, wherein the antibody is a polyclonal antibody or monoclonal  
34            antibody.

35        17. A pharmaceutical composition comprising the agent of claim 12 and a  
36            pharmaceutically acceptable carrier.

37        18. A method for modulating a mNa<sub>v</sub>1.3  $\alpha$  subunit polypeptide activity in a cell, the  
38            method comprising:  
39                    providing a sodium channel comprising a mNa<sub>v</sub>1.3  $\alpha$  subunit polypeptide,  
40                    wherein the mNa<sub>v</sub>1.3  $\alpha$  subunit polypeptide is according to any of claims 1-3;  
41                    contacting the channel with an amount of a mNa<sub>v</sub>1.3  $\alpha$  subunit polypeptide  
42                    modulator effective to modulate an activity of the mNa<sub>v</sub>1.3  $\alpha$  subunit polypeptide.

43        19. The method of claim 18, wherein the modulator is a small molecule, a nucleic acid, or  
44            a protein.

45        20. A method for identifying an agent that modulates the activity of a mNa<sub>v</sub>1.3  $\alpha$  subunit  
46            polypeptide, the method comprising:  
47                    providing a first sodium channel comprising a mNa<sub>v</sub>1.3  $\alpha$  subunit  
48                    polypeptide, wherein the a mNa<sub>v</sub>1.3  $\alpha$  subunit polypeptide is according to any of  
49                    claims 1-3;  
50                    contacting the channel with a test compound; and  
51                    evaluating an activity of the sodium channel, wherein a change in activity  
52                    relative to a reference value is an indication that the compound is an agent that  
53                    modulates the channel.

54        21. The method of claim 20, wherein the test compound is a small molecule, a peptide, or  
55            a nucleic acid.

56        22. The method of claim 20, wherein the sodium channel is contained within a biological  
57            sample.

58        23. The method of claim 20, wherein the channel is contacted with multiple test  
59            compounds.

60           24. The method of claim 20, wherein the sample comprises a cell membrane.  
61           25. The method of claim 24, wherein the sample comprises a cell.  
62           26. The method of claim 25, wherein the cell is a eukaryotic cell.  
63           27. The method of claim 26, wherein the cell is *Xenopus* oocyte.  
64           28. The method of claim 26, wherein the cell is a mammalian cell.  
65           29. The method of claim 20, wherein the activity comprises regulation of sodium  
66           concentration.  
67           30. The method of claim 20, wherein the evaluating comprises detecting sodium flux.  
68           31. The method of claim 20, wherein the contacting occurs under conditions which, in the  
69           absence of the test compound, cause a first amount of sodium flux.  
70           32. The method of claim 20, wherein the evaluating comprises using a  $\text{Na}^+$  flux assay.  
71           33. The method of claim 20, wherein the assay uses patch clamp electrophysiology.  
72           34. The method of claim 20, wherein the assay uses two electrode voltage clamp  
73           electrophysiology.  
74           35. The method of claim 20, wherein the assay comprises using a sodium-sensitive dye.  
75           36. The method of claim 20, wherein the assay is a high-throughput assay.  
76           37. The method of claim 20, further comprising the steps of:  
77                   providing a second sodium channel comprising a  $\text{mNa}_v1.3 \alpha$  subunit  
78                   polypeptide, wherein the  $\text{mNa}_v1.3 \alpha$  subunit polypeptide is other than a  $\text{mNa}_v1.3 \alpha$   
79                   subunit polypeptide according to claims 1-3;  
80                   contacting the second sodium channel with the test compound;  
81                   evaluating the activity of the second sodium channel.  
82           38. The method of claim 37, further comprising comparing the activity of the first sodium  
83           channel in the presence of the test compound to the activity of the second sodium  
84           channel in the presence of the test compound.  
85           39. The method of claim 37, wherein a plurality of sodium channels are provided.  
86           40. The method of claim 20, wherein the  $\text{mNa}_v1.3 \alpha$  subunit polypeptide comprises the  
87           amino acid sequence of SEQ ID NO:2.  
88           41. A method for identifying an agent useful in the treatment of a disorder related to  
89           sodium current modulation, the method comprising:  
90                   providing a sodium channel comprising a  $\text{mNa}_v1.3 \alpha$  subunit polypeptide

91 according to any of claims 1-3;

92 contacting the channel with a test compound; and

93 evaluating an activity of the channel, wherein a change in activity relative to a

94 reference value is an indication that the test compound is an agent useful in a disorder

95 related to sodium current.

96 42. The method of claim 41, wherein the disorder is pain, paraesthesia, stroke, head

97 trauma, a neurodegenerative disorder, or a disorder related to hyperexcitability of

98 neurons.

99 43. The method of claim 41, further comprising administering the compound *in vivo*.

100 44. The method of claim 41, further comprising modifying the compound for use *in vivo*.

101 45. The method of claim 41, further comprising evaluating modulation of a related

102 human  $\text{Na}_v1.3$   $\alpha$  subunit polypeptide by the compound.

103 46. A method for treating a subject having a disorder related to sodium channel current,

104 the method comprising:

105 identifying an agent that selectively binds a  $\text{mNa}_v1.3$   $\alpha$  subunit polypeptide,

106 and

107 administering to a subject in need of such treatment a pharmacological agent

108 which is selective for a sodium channel comprising a  $\text{mNa}_v1.3$   $\alpha$  subunit polypeptide.

109 47. The method of claim 46, wherein the disorder is pain, paraesthesia, stroke, head

110 trauma, a neurodegenerative disorder, or a disorder related to hyperexcitability of

111 neurons.

112

113

**ABSTRACT**

Novel sodium channel nucleic acids and polypeptides are disclosed herein. Methods of using the novel nucleic acids and polypeptides are also disclosed.

20571493.doc

This Page Is Inserted by IFW Operations  
and is not a part of the Official Record

## **BEST AVAILABLE IMAGES**

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

**IMAGES ARE BEST AVAILABLE COPY.**

**As rescanning documents *will not* correct images,  
please do not report the images to the  
Problem Image Mailbox.**

# FIG. 1

## PROTEIN ALIGNMENT OF MOUSE, HUMAN AND RAT Nav1.3

mNav1.3 wild-type	(1)	MAGALLVPPGPISTERFRESLAIKRAAEKAKPKKEQDIDDENPKK
hNav1.3 NM_006922	(1)	MAGALLVPPGPISTERFRESLAIKRAAEKAKPKKEQDIDDENPKK
hNav1.3 AF225986	(1)	MAGALLVPPGPISTERFRESLAIKRAAEKAKPKKEQDIDDENPKK
rNav1.3 NM_013119	(1)	MAGALLVPPGPISTERFRESLAIKRAAEKAKPKKEQDIDDENPKK
mNav1.3 wild-type	(51)	PSDLEAGANLPLFYGDIPIPENNSELEDDPYYKSKTLVWVNGKAI
hNav1.3 NM_006922	(51)	PSDLEAGANLPLFYGDIPIPENNSELEDDPYYKSKTLVWVNGKAI
hNav1.3 AF225986	(51)	PSDLEAGANLPLFYGDIPIPENNSELEDDPYYKSKTLVWVNGKAI
rNav1.3 NM_013119	(51)	PSDLEAGANLPLFYGDIPIPENNSELEDDPYYKSKTLVWVNGKAI
mNav1.3 wild-type	(101)	RESAATLYLPLNVRKIAKILHSLFSMIMCTLTNCMFLMSNP
hNav1.3 NM_006922	(101)	RESAATLYLPLNVRKIAKILHSLFSMIMCTLTNCMFLMSNP
hNav1.3 AF225986	(101)	RESAATLYLPLNVRKIAKILHSLFSMIMCTLTNCMFLMSNP
rNav1.3 NM_013119	(101)	RESAATLYLPLNVRKIAKILHSLFSMIMCTLTNCMFLMSNP
mNav1.3 wild-type	(151)	PDTRKVEVTFGIGTEFESLKLARGECLDEDFTLRDPNWIDFSTVW
hNav1.3 NM_006922	(151)	PDTRKVEVTFGIGTEFESLKLARGECLDEDFTLRDPNWIDFSTVW
hNav1.3 AF225986	(151)	PDTRKVEVTFGIGTEFESLKLARGECLDEDFTLRDPNWIDFSTVW
rNav1.3 NM_013119	(151)	PDTRKVEVTFGIGTEFESLKLARGECLDEDFTLRDPNWIDFSTVW
mNav1.3 wild-type	(201)	AVVTEFDLGNSVSLTTERFLRALKTISVPGKLTGALIOSVKKLSDV
hNav1.3 NM_006922	(201)	AVVTEFDLGNSVSLTTERFLRALKTISVPGKLTGALIOSVKKLSDV
hNav1.3 AF225986	(201)	AVVTEFDLGNSVSLTTERFLRALKTISVPGKLTGALIOSVKKLSDV
rNav1.3 NM_013119	(201)	AVVTEFDLGNSVSLTTERFLRALKTISVPGKLTGALIOSVKKLSDV
mNav1.3 wild-type	(251)	MITYFGLSVEALTFGQLPGLRURGECPWPSDSEAFNTSYENGMD
hNav1.3 NM_006922	(251)	MITYFGLSVEALTFGQLPGLRURGECPWPSDSEAFNTSYENGMD
hNav1.3 AF225986	(251)	MITYFGLSVEALTFGQLPGLRURGECPWPSDSEAFNTSYENGMD
rNav1.3 NM_013119	(251)	MITYFGLSVEALTFGQLPGLRURGECPWPSDSEAFNTSYENGMD

mNav1.3 wild-type	(301)	SGTTEVNIMSTENWWDY	DDSHFTYLDQDQRPLLGNSDAGCPEGT
hNav1.3 NM_006922	(301)	SGTTEVNIMSTENWWDY	DDSHFTYLDQDQRPLLGNSDAGCPEGT
hNav1.3 AF225986	(301)	SGTTEVNIMSTENWWDY	DDSHFTYLDQDQRPLLGNSDAGCPEGT
rNav1.3 NM_013119	(301)	SGTTEVNIMSTENWWDY	DDSHFTYLDQDQRPLLGNSDAGCPEGT
mNav1.3 wild-type	(351)	TCVAGRNINYGTSIDTFSWALSFRMTQYDWNLYQLTIRAGKT	
hNav1.3 NM_006922	(351)	TCVAGRNINYGTSIDTFSWALSFRMTQYDWNLYQLTIRAGKT	
hNav1.3 AF225986	(351)	TCVAGRNINYGTSIDTFSWALSFRMTQYDWNLYQLTIRAGKT	
rNav1.3 NM_013119	(351)	TCVAGRNINYGTSIDTFSWALSFRMTQYDWNLYQLTIRAGKT	
mNav1.3 wild-type	(401)	MFEFWVVIIFLGSYLVNLIAVAMYEQNLQATLEAFQKEAEFOOMIE	
hNav1.3 NM_006922	(401)	MFEFWVVIIFLGSYLVNLIAVAMYEQNLQATLEAFQKEAEFOOMIE	
hNav1.3 AF225986	(401)	MFEFWVVIIFLGSYLVNLIAVAMYEQNLQATLEAFQKEAEFOOMIE	
rNav1.3 NM_013119	(401)	MFEFWVVIIFLGSYLVNLIAVAMYEQNLQATLEAFQKEAEFOOMIE	
mNav1.3 wild-type	(451)	QEKQOEAOAABAAARDESQ	GGQBLIESSSEAASKSSKAKER
hNav1.3 NM_006922	(451)	QEKQOEAOAABAAARDESQ	GGQBLIESSSEAASKSSKAKER
hNav1.3 AF225986	(451)	QEKQOEAOAABAAARDESQ	GGQBLIESSSEAASKSSKAKER
rNav1.3 NM_013119	(451)	QEKQOEAOAABAAARDESQ	GGQBLIESSSEAASKSSKAKER
mNav1.3 wild-type	(501)	NRKRRQREHLEGH	PGDREPKESEDSUKRSFILSDONPFDGDR
hNav1.3 NM_006922	(501)	NRKRRQREHLEGH	PGDREPKESEDSUKRSFILSDONPFDGDR
hNav1.3 AF225986	(501)	NRKRRQREHLEGH	PGDREPKESEDSUKRSFILSDONPFDGDR
rNav1.3 NM_013119	(501)	NRKRRQREHLEGH	PGDREPKESEDSUKRSFILSDONPFDGDR
mNav1.3 wild-type	(551)	CSPHOSLSTRGSLFSPRRNSKTSI	FSFRERAKOVSSENDADEHST
hNav1.3 NM_006922	(551)	CSPHOSLSTRGSLFSPRRNSKTSI	FSFRERAKOVSSENDADEHST
hNav1.3 AF225986	(551)	CSPHOSLSTRGSLFSPRRNSKTSI	FSFRERAKOVSSENDADEHST
rNav1.3 NM_013119	(551)	CSPHOSLSTRGSLFSPRRNSKTSI	FSFRERAKOVSSENDADEHST
mNav1.3 wild-type	(601)	FSRSRDSLEPPIRE	GERRNSNSGSGSSTCAGT
hNav1.3 NM_006922	(601)	FSRSRDSLEPPIRE	GERRNSNSGSGSSTCAGT
hNav1.3 AF225986	(601)	FSRSRDSLEPPIRE	GERRNSNSGSGSSTCAGT
rNav1.3 NM_013119	(601)	FSRSRDSLEPPIRE	GERRNSNSGSGSSTCAGT

mNav1.3	wild-type	(651)	YQYS-----STTTTEVKKRKLSSQYQSMIMLDDSS
hNav1.3	NM_006922	(651)	YQPS-----STTTTEVKKRKLSSQYQSMIMLDDSS
hNav1.3	AF225986	(625)	YQPS-----STTTTEVKKRKLSSQYQSMIMLDDSS
rNav1.3	NM_013119	(625)	YQPS-----STTTTEVKKRKLSSQYQSMIMLDDSS
mNav1.3	wild-type	(684)	GROPS-----SIASTLTNTMMEELIESROKCPYKRYANVLEINDDOCCQSMKVK
hNav1.3	NM_006922	(701)	GROPS-----SIASTLTNTMMEELIESROKCPYKRYANVLEINDDOCCQSMKVK
hNav1.3	AF225986	(652)	GROPS-----SIASTLTNTMMEELIESROKCPYKRYANVLEINDDOCCQSMKVK
rNav1.3	NM_013119	(652)	GROPS-----SIASTLTNTMMEELIESROKCPYKRYANVLEINDDOCCQSMKVK
mNav1.3	wild-type	(734)	HLVNLVNDREPDVIAITCIVLNFAMEMHYPPTDQSSTVLTGVNLVFT
hNav1.3	NM_006922	(751)	HLVNLVNDREPDVIAITCIVLNFAMEMHYPPTDQSSTVLTGVNLVFT
hNav1.3	AF225986	(702)	HLVNLVNDREPDVIAITCIVLNFAMEMHYPPTDQSSTVLTGVNLVFT
rNav1.3	NM_013119	(702)	HLVNLVNDREPDVIAITCIVLNFAMEMHYPPTDQSSTVLTGVNLVFT
mNav1.3	wild-type	(784)	GTTFPAAEMKLTANDPPIYDQEGNNTIDGTIUSLSEMEIGL-----NVEGUSV
hNav1.3	NM_006922	(801)	GTTFPAAEMKLTANDPPIYDQEGNNTIDGTIUSLSEMEIGL-----NVEGUSV
hNav1.3	AF225986	(752)	GTTFPAAEMKLTANDPPIYDQEGNNTIDGTIUSLSEMEIGL-----NVEGUSV
rNav1.3	NM_013119	(752)	GTTFPAAEMKLTANDPPIYDQEGNNTIDGTIUSLSEMEIGL-----NVEGUSV
mNav1.3	wild-type	(834)	RSFRILRVEKIAKSPNMLKIGNSVAGENIPLVLTALMEIFAVG
hNav1.3	NM_006922	(851)	RSFRILRVEKIAKSPNMLKIGNSVAGENIPLVLTALMEIFAVG
hNav1.3	AF225986	(802)	RSFRILRVEKIAKSPNMLKIGNSVAGENIPLVLTALMEIFAVG
rNav1.3	NM_013119	(802)	RSFRILRVEKIAKSPNMLKIGNSVAGENIPLVLTALMEIFAVG
mNav1.3	wild-type	(884)	MOLFQSKYKECVCTINDCQKPLWHNDDEHFSUPLVRVCGEMIETMWD
hNav1.3	NM_006922	(901)	MOLFQSKYKECVCTINDCQKPLWHNDDEHFSUPLVRVCGEMIETMWD
hNav1.3	AF225986	(852)	MOLFQSKYKECVCTINDCQKPLWHNDDEHFSUPLVRVCGEMIETMWD
rNav1.3	NM_013119	(852)	MOLFQSKYKECVCTINDCQKPLWHNDDEHFSUPLVRVCGEMIETMWD
mNav1.3	wild-type	(934)	CMEVAGCMMCTVMEVMMAGNLYVNLFLAIISSSSENLLATDDDN
hNav1.3	NM_006922	(902)	CMEVAGCMMCTVMEVMMAGNLYVNLFLAIISSSSENLLATDDDN
hNav1.3	AF225986	(902)	CMEVAGCMMCTVMEVMMAGNLYVNLFLAIISSSSENLLATDDDN
rNav1.3	NM_013119	(902)	CMEVAGCMMCTVMEVMMAGNLYVNLFLAIISSSSENLLATDDDN

mNav1.3 wild-type	(984)	MNINLOIAGEMGOKGIVIVKNAKRECHKAFFKRPKVTEFEGAKTDSCHS
hNav1.3 NM_006922	(1001)	MNINLOIAGEMGOKGIVIVKNAKRECHKAFFKRPKVTEFEGAKTDSCHS
hNav1.3 AF225986	(952)	MNINLOIAGEMGOKGIVIVKNAKRECHKAFFKRPKVTEFEGAKTDSCHS
rNav1.3 NM_013119	(952)	MNINLOIAGEMGOKGIVIVKNAKRECHKAFFKRPKVTEFEGAKTDSCHS
mNav1.3 wild-type	(1034)	NNTCGKISKEINLYLDGNGTSGCAGTGSSEKVKIDENDYMFINNPSE
hNav1.3 NM_006922	(1051)	NNTCGKISKEINLYLDGNGTSGCAGTGSSEKVKIDENDYMFINNPSE
hNav1.3 AF225986	(1002)	NNTCGKISKEINLYLDGNGTSGCAGTGSSEKVKIDENDYMFINNPSE
rNav1.3 NM_013119	(1002)	NNTCGKISKEINLYLDGNGTSGCAGTGSSEKVKIDENDYMFINNPSE
mNav1.3 wild-type	(1084)	TVVIVPTAVGESDENANTFEESESESTIESKERNLNTSSSESTSTVVA
hNav1.3 NM_006922	(1100)	TVVIVPTAVGESDENANTFEESESESTIESKERNLNTSSSESTSTVVA
hNav1.3 AF225986	(1051)	TVVIVPTAVGESDENANTFEESESESTIESKERNLNTSSSESTSTVVA
rNav1.3 NM_013119	(1051)	TVVIVPTAVGESDENANTFEESESESTIESKERNLNTSSSESTSTVVA
mNav1.3 wild-type	(1134)	RECOEAEIREEEKAPECACTEGCIIKKPFCOVSBEGRGKIKWNLKTC
hNav1.3 NM_006922	(1150)	RECOEAEIREEEKAPECACTEGCIIKKPFCOVSBEGRGKIKWNLKTC
hNav1.3 AF225986	(1101)	RECOEAEIREEEKAPECACTEGCIIKKPFCOVSBEGRGKIKWNLKTC
rNav1.3 NM_013119	(1101)	RECOEAEIREEEKAPECACTEGCIIKKPFCOVSBEGRGKIKWNLKTC
mNav1.3 wild-type	(1184)	YSVIENNWETTIVFALISSLSSGALAFEDTYIORKTKIKMLEYADKVFY
hNav1.3 NM_006922	(1200)	YSVIENNWETTIVFALISSLSSGALAFEDTYIORKTKIKMLEYADKVFY
hNav1.3 AF225986	(1151)	YSVIENNWETTIVFALISSLSSGALAFEDTYIORKTKIKMLEYADKVFY
rNav1.3 NM_013119	(1151)	YSVIENNWETTIVFALISSLSSGALAFEDTYIORKTKIKMLEYADKVFY
mNav1.3 wild-type	(1234)	TEILEMELIKWAVGFTYIYNACWMDFLIVDVSLSVLANALGYSSELGA
hNav1.3 NM_006922	(1250)	TEILEMELIKWAVGFTYIYNACWMDFLIVDVSLSVLANALGYSSELGA
hNav1.3 AF225986	(1201)	TEILEMELIKWAVGFTYIYNACWMDFLIVDVSLSVLANALGYSSELGA
rNav1.3 NM_013119	(1201)	TEILEMELIKWAVGFTYIYNACWMDFLIVDVSLSVLANALGYSSELGA
mNav1.3 wild-type	(1284)	TKSLTRLRLPRLSREGRVRVNAVALGAPSIMNVLQCLLIE
hNav1.3 NM_006922	(1300)	TKSLTRLRLPRLSREGRVRVNAVALGAPSIMNVLQCLLIE
hNav1.3 AF225986	(1251)	TKSLTRLRLPRLSREGRVRVNAVALGAPSIMNVLQCLLIE
rNav1.3 NM_013119	(1251)	TKSLTRLRLPRLSREGRVRVNAVALGAPSIMNVLQCLLIE

mNav1.3 wild-type	(1334)	SINGNLLAGKEHCVNNFTGSMFDSSEVNNFSDCOALGQAOAWKVKVN
hNav1.3 NM_006922	(1350)	SINGNLLAGKEHCVNNFTGSMFDSSEVNNFSDCOALGQAOAWKVKVN
hNav1.3 AF225986	(1301)	SINGNLLAGKEHCVNNFTGSMFDSSEVNNFSDCOALGQAOAWKVKVN
rNav1.3 NM_013119	(1301)	SINGNLLAGKEHCVNNFTGSMFDSSEVNNFSDCOALGQAOAWKVKVN
mNav1.3 wild-type	(1384)	ENUGAGYLALIQLQAFKQWMTMKAADSRSOKLOPVEENLYMLYF
hNav1.3 NM_006922	(1400)	ENUGAGYLALIQLQAFKQWMTMKAADSRSOKLOPVEENLYMLYF
hNav1.3 AF225986	(1351)	ENUGAGYLALIQLQAFKQWMTMKAADSRSOKLOPVEENLYMLYF
rNav1.3 NM_013119	(1351)	ENUGAGYLALIQLQAFKQWMTMKAADSRSOKLOPVEENLYMLYF
mNav1.3 wild-type	(1434)	TEIIGESFTETLNUFQIVDNEQOKKEFQGDIYFTEOKKYNNAMKKI
hNav1.3 NM_006922	(1450)	TEIIGESFTETLNUFQIVDNEQOKKEFQGDIYFTEOKKYNNAMKKI
hNav1.3 AF225986	(1401)	TEIIGESFTETLNUFQIVDNEQOKKEFQGDIYFTEOKKYNNAMKKI
rNav1.3 NM_013119	(1401)	TEIIGESFTETLNUFQIVDNEQOKKEFQGDIYFTEOKKYNNAMKKI
mNav1.3 wild-type	(1484)	GSKRDKPDKPTPREPKNSQGRVNEFVTQFDFDTSIMLUGANNMVTMDD
hNav1.3 NM_006922	(1500)	GSKKPKPDKPTPREPKNSQGRVNEFVTQFDFDTSIMLUGANNMVTMDD
hNav1.3 AF225986	(1451)	GSKRDKPDKPTPREPKNSQGRVNEFVTQFDFDTSIMLUGANNMVTMDD
rNav1.3 NM_013119	(1451)	GSKKPKPDKPTPREPKNSQGRVNEFVTQFDFDTSIMLUGANNMVTMDD
mNav1.3 wild-type	(1534)	QKRYMFLVLSRNLNVIVLFTEFKLTLSRSLYYETTIGNNEDFVVVII
hNav1.3 NM_006922	(1550)	QKRYMFLVLSRNLNVIVLFTEFKLTLSRSLYYETTIGNNEDFVVVII
hNav1.3 AF225986	(1501)	QKRYMFLVLSRNLNVIVLFTEFKLTLSRSLYYETTIGNNEDFVVVII
rNav1.3 NM_013119	(1501)	QKRYMFLVLSRNLNVIVLFTEFKLTLSRSLYYETTIGNNEDFVVVII
mNav1.3 wild-type	(1584)	SIVGMFLAELIETKPSPLFMRVIRARIGRLRJKGKGKIRTLLFALQ
hNav1.3 NM_006922	(1600)	SIVGMFLAELIETKPSPLFMRVIRARIGRLRJKGKGKIRTLLFALQ
hNav1.3 AF225986	(1551)	SIVGMFLAELIETKPSPLFMRVIRARIGRLRJKGKGKIRTLLFALQ
rNav1.3 NM_013119	(1551)	SIVGMFLAELIETKPSPLFMRVIRARIGRLRJKGKGKIRTLLFALQ
mNav1.3 wild-type	(1634)	MSPALPENGLLFLVMEYAFGNSNFAYVKKEAGIDMENEFEGNSP
hNav1.3 NM_006922	(1650)	MSPALPENGLLFLVMEYAFGNSNFAYVKKEAGIDMENEFEGNSP
hNav1.3 AF225986	(1601)	MSPALPENGLLFLVMEYAFGNSNFAYVKKEAGIDMENEFEGNSP
rNav1.3 NM_013119	(1601)	MSPALPENGLLFLVMEYAFGNSNFAYVKKEAGIDMENEFEGNSP

mNav1.3	wild-type	(1.684)	ICLFOQTISAGAGGLAPLINSAPPDQDPAIPCGSSVAKGDC	PSVGI
hNav1.3	NM_006922	(1.700)	ICLFOQTISAGAGGLAPLINSAPPDQDPAIPCGSSVAKGDC	PSVGI
hNav1.3	AF225986	(1.651)	ICLFOQTISAGAGGLAPLINSAPPDQDPAIPCGSSVAKGDC	PSVGI
rNav1.3	NM_013119	(1.651)	ICLFOQTISAGAGGLAPLINSAPPDQDPAIPCGSSVAKGDC	PSVGI
mNav1.3	wild-type	(1.734)	FEVSYLITSELMWVNNYIATVLENSEVAESELESEDEMEYEVW	
hNav1.3	NM_006922	(1.750)	FEVSYLITSELMWVNNYIATVLENSEVAESELESEDEMEYEVW	
hNav1.3	AF225986	(1.701)	FEVSYLITSELMWVNNYIATVLENSEVAESELESEDEMEYEVW	
rNav1.3	NM_013119	(1.701)	FEVSYLITSELMWVNNYIATVLENSEVAESELESEDEMEYEVW	
mNav1.3	wild-type	(1.784)	FDPDATOFECKLDFEADALPPLIATPKNQVLIAMOLPMSGDRTHC	
hNav1.3	NM_006922	(1.800)	FDPDATOFECKLDFEADALPPLIATPKNQVLIAMOLPMSGDRTHC	
hNav1.3	AF225986	(1.751)	FDPDATOFECKLDFEADALPPLIATPKNQVLIAMOLPMSGDRTHC	
rNav1.3	NM_013119	(1.751)	FDPDATOFECKLDFEADALPPLIATPKNQVLIAMOLPMSGDRTHC	
mNav1.3	wild-type	(1.834)	DOTLPAFTKRVVSESEGMDALIOMEDRIMASNPVSVEPITITLKRK	
hNav1.3	NM_006922	(1.850)	DOTLPAFTKRVVSESEGMDALIOMEDRIMASNPVSVEPITITLKRK	
hNav1.3	AF225986	(1.801)	DOTLPAFTKRVVSESEGMDALIOMEDRIMASNPVSVEPITITLKRK	
rNav1.3	NM_013119	(1.801)	DOTLPAFTKRVVSESEGMDALIOMEDRIMASNPVSVEPITITLKRK	
mNav1.3	wild-type	(1.884)	EVVSAITLORVRCILKORLENISKQKEIKR1VPIFGDK	VIDR
hNav1.3	NM_006922	(1.900)	EVVSAITLORVRCILKORLENISKQKEAKR1VPIFGDK	VIDR
hNav1.3	AF225986	(1.851)	EVVSAITLORVRCILKORLENISKQKEAKR1VPIFGDK	VIDR
rNav1.3	NM_013119	(1.851)	EVVSAITLORVRCILKORLENISKQKEIKR1VPIFGDK	VIDR
mNav1.3	wild-type	(1.934)	GENISPERKTDGSSSTTPPSYDOSVTPDEKEFKDKEPEKPSCKEV	--
hNav1.3	NM_006922	(1.950)	GENISPERKTDGSSSTTPPSYDOSVTPDEKEFKDKEPEKPSCKEV	--
hNav1.3	AF225986	(1.901)	GENISPERKTDGSSSTTPPSYDOSVTPDEKEFKDKEPEKPSCKEV	--
rNav1.3	NM_013119	(1.901)	GENISPERKTDGSSSTTPPSYDOSVTPDEKEFKDKEPEKPSCKEV	--
mNav1.3	wild-type	(1.980)	--	
hNav1.3	NM_006922	(2000)	--	
hNav1.3	AF225986	(1951)	--	
rNav1.3	NM_013119	(1951)	--	

## FIG. 2

### ALIGNMENT OF FULL-LENGTH AND PARTIAL MOUSE Nav1.3 PROTEIN

mNav1.3 wild-type	(1)	MAQALLVPPGPESFRLFTRESLAAIEKRAAEKAKKPKE
mNav1.3 NM_018732		-----
mNav1.3 wild-type	(41)	QDIDDENKPKPNSDLEAGKNLPIYGDIPPEMVSEPLEDL
mNav1.3 NM_018732		-----
mNav1.3 wild-type	(81)	DPYVVSKKTFVVLNKGKAIFRFSATSALYILTPLNPVRKI
mNav1.3 NM_018732		-----
mNav1.3 wild-type	(121)	AIKILVHSLFSMLIMCTILTNCFMTLSNPWDTKNVEYT
mNav1.3 NM_018732		-----
mNav1.3 wild-type	(161)	FTGIYTFESLIKILARGFCLEDFTFLRDPWNWLDFSVIVM
mNav1.3 NM_018732		-----
mNav1.3 wild-type	(201)	AYVTEFVDLGNVSAALRTFRVLRALKTISVPIGLKTIVGAL
mNav1.3 NM_018732		-----
mNav1.3 wild-type	(241)	IQSVKKLSDVMILTVFCLSVFALIGLQLFMGNLNRNKCLQW
mNav1.3 NM_018732		-----
mNav1.3 wild-type	(281)	PPSDSAFEINTTSYFNGTMDSNGTFVNVTMSTFNWKDYIA
mNav1.3 NM_018732		-----
mNav1.3 wild-type	(321)	DDSHFYVLDGQKDPLLCGNNSDAGQCPEGYICVKAGRNPN
mNav1.3 NM_018732		-----
mNav1.3 wild-type	(361)	YGYTSFDTFSWAFSLSLFRIMTQDYWENLYQLTLRAAGKTY
mNav1.3 NM_018732		-----
mNav1.3 wild-type	(401)	MIFFVLVIFLGSFYLVNLILAVVAMAYEEQNQATLEEAQ
mNav1.3 NM_018732		-----
mNav1.3 wild-type	(441)	KEAEFQQMLEQLKKQQEEAQAVAAAASAASRDFSGIGGLGE
mNav1.3 NM_018732		-----
mNav1.3 wild-type	(481)	LLESSSEASKLSSKSAKEWRNRRKKRQREHLEGNHRPEG
mNav1.3 NM_018732		-----
mNav1.3 wild-type	(521)	DRFPKSESEDSVKKRSFLSDGNPLSGDKKLCSPHQSSL
mNav1.3 NM_018732		-----
mNav1.3 wild-type	(561)	SIRGSLFSPRRNSKTSIFSRGRAKDVGSENDFADDEHST
mNav1.3 NM_018732		-----
mNav1.3 wild-type	(601)	FEDSESRDLSLFVPHRGERRNSNVSQASMSSRMVPGPA
mNav1.3 NM_018732		-----
mNav1.3 wild-type	(641)	NGKMHSTVDCNGVVSLGTTETEVRKRRLLSSYQISMEMLE
mNav1.3 NM_018732		-----

mnNav1.3 wild-type mnNav1.3 NM_018732	(681) DSSGRQRAMSIASILTNTMEELEESRQKCPCWYRFANVF -----
mnNav1.3 wild-type mnNav1.3 NM_018732	(721) LIWDCCDSWLKVKHLVNLIVMDPFVDLAITICIVLNTLFM -----
mnNav1.3 wild-type mnNav1.3 NM_018732	(761) AMEHYPMTEQFSSVLTVGVLVFTGIFTAEMVLKIIAMDHY -----
mnNav1.3 wild-type mnNav1.3 NM_018732	(801) YYFQEGWNIFDGIIVSLSLMEGLANVEGLSVLRSFRLLR -----
mnNav1.3 wild-type mnNav1.3 NM_018732	(841) VFKLAKSWPTLNMLKIIIGNSVGALGNLTIVLAIIVF1FA (1) -----MIIKIGNSVGALGNLTIVLAIIVF1FA
mnNav1.3 wild-type mnNav1.3 NM_018732	(881) VVGMQLEGKSYKECVKINEDCKLPRWHMNDFFHSEFLIVE (29) VVGMQLEGKSYKECVKINEDCKLERWHMNDFFHSEFLIVE
mnNav1.3 wild-type mnNav1.3 NM_018732	(921) RVLCGWEIETMWDCMEVAGQIMCLIVFMLVMVIGNLWLN (69) RVLCGWEIETMWDCMEVAGQIMCLIVFMLVMVIGNLWLN
mnNav1.3 wild-type mnNav1.3 NM_018732	(961) LFLAIIILSSSESSDNLAATDDNEEMNNLQIAVGRMOKGIDY (109) LFLAIIILSSSESSDNLAATDDNEEMNNLQIAVGRMOKGIDY
mnNav1.3 wild-type mnNav1.3 NM_018732	(1001) VVNNKIRECFFKAERPKVIEIHEGNKIDSCMSNNTGVVE (149) VVNNKIRECFFKAERPKVIEIHEGNKIDSCMSNNTGVVE
mnNav1.3 wild-type mnNav1.3 NM_018732	(1041) ISKELNYLKDGNNTTSGVGTSVKEVYVIDENDYMSFINN (189) ISKELNYLKDGNNTTSGVGTSVKEVYVIDENDYMSFINN
mnNav1.3 wild-type mnNav1.3 NM_018732	(1081) PSETVTVPIAVGESDFENLNTEEFSSSELEESKEKLNT (229) PSETVTVPIAVGESDFENLNTEEFSSSELEESKEVSALF
mnNav1.3 wild-type mnNav1.3 NM_018732	(1121) SSSEGSTVDVAPPREGEQAEPEEEDLKPEACFTEGCIKK (269) SSGHHCFAIYTWLLCIVFC-----
mnNav1.3 wild-type mnNav1.3 NM_018732	(1161) FPFCQVSTEEGKGKIIWWNLRKTCYSIVEHNWFETFIVFMI -----
mnNav1.3 wild-type mnNav1.3 NM_018732	(1201) LLSSGALAFEDIYIEQRKTIKTMLEYADKVFTYIFILEML -----
mnNav1.3 wild-type mnNav1.3 NM_018732	(1241) LKWVWAYGFQTYFTNAWCWLDFLIVDVSLSVSLVANALGYSE -----
mnNav1.3 wild-type mnNav1.3 NM_018732	(1281) LGAIKSLRTLRLRPLRALSRFEGMRVVNVNALVGAIPSIM -----
mnNav1.3 wild-type mnNav1.3 NM_018732	(1321) NVLLVCLIFWLIFSIMGVNLFAGKFYHCVNMTGSMFDMS -----
mnNav1.3 wild-type mnNav1.3 NM_018732	(1361) EVNNFSDCQALGKQARWKNVKVNFDNVGAGYLALLQVATF -----
mnNav1.3 wild-type mnNav1.3 NM_018732	(1401) KGWMMDIMYAAVDSRDVKLQPVYEEENLYMLYFVIFIIFGS -----

mNav1.3 wild-type (1441) FFTLNLFIGVIIDNFNQQKKFGGQDIFMTEEQKYYNAM  
mNav1.3 NM\_018732 -----

mNav1.3 wild-type (1481) KKLGSKKPQKPIPRAKPFQGMVFDFVTRQVFDISIMILI  
mNav1.3 NM\_018732 -----

mNav1.3 wild-type (1521) CLNMVTMMVETDDQSKYMTLVLRSRINLVFIVLFTGEFLKK  
mNav1.3 NM\_018732 -----

mNav1.3 wild-type (1561) LISLRYYYFTIGWNIFDFVVVILSIVGMFLAELIEKYFVS  
mNav1.3 NM\_018732 -----

mNav1.3 wild-type (1601) PTLFRVIRLARIGRILRLIKGAKGIRTLFLALMMSLPALF  
mNav1.3 NM\_018732 -----

mNav1.3 wild-type (1641) NIGLLLFLVMFIYAIIFGMSNFAYVKKEAGIDDMFNFETFG  
mNav1.3 NM\_018732 -----

mNav1.3 wild-type (1681) NSMICLFQITTSAGWDGLLAPILNSAPPDCDPDAIHPGSS  
mNav1.3 NM\_018732 -----

mNav1.3 wild-type (1721) VKGDCGNPSVGIFFFVSYIIISFLVVVNMYIAVILENFV  
mNav1.3 NM\_018732 -----

mNav1.3 wild-type (1761) ATEESAEPLESEDDFEMFYEVWEKFDPDATQFIEFCKLSDF  
mNav1.3 NM\_018732 -----

mNav1.3 wild-type (1801) AAALDPPLIARIKPNKVQLIAMDLPMVSGDRIHCLDILFAF  
mNav1.3 NM\_018732 -----

mNav1.3 wild-type (1841) TKRVLGESGEMDALRIQMEDRFMASNPSKVSYEPITTLK  
mNav1.3 NM\_018732 -----

mNav1.3 wild-type (1881) RKQEEVSAIIQRNYRCYLLKQRLKNISNTYDKEIKGRI  
mNav1.3 NM\_018732 -----

mNav1.3 wild-type (1921) VLPIKGDMVIDKLNGNSTPEKTDGSSTTSPPSYDSVTKP  
mNav1.3 NM\_018732 -----

mNav1.3 wild-type (1961) DKEKFEKDKPEKESKGKEV█  
mNav1.3 NM\_018732 -----

FIG. 3

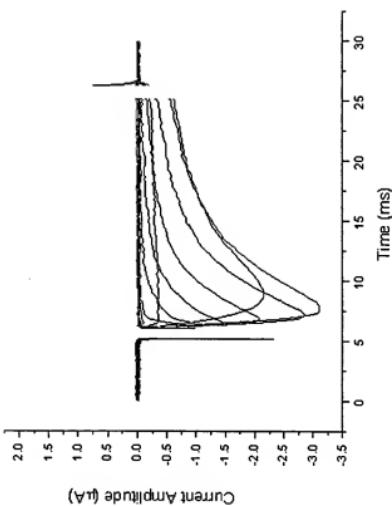


FIG. 4

